MONASH University

Optimising Bone and Reproductive Health in Individuals with Type 1 Diabetes

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Bachelor of Medicine and Surgery (Hons)

A thesis submitted in fulfilment of the requirements
for the degree of

Doctor of Philosophy

Monash University 2020

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GENERAL DECLARATION FOR THESIS INCLUDING PUBLISHED WORKS

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 4 original papers published in peer reviewed journals and 2 publications currently in review. There is an additional publication within the supplemental files.

The core theme of the thesis examines the relationships between type 1 diabetes with bone and reproductive health. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, Monash University, under the supervision of Professor Helena Teede, Associate Professor Frances Milat and Professor Peter Ebeling.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapter 1 to 4, my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status *(published, in press, accepted or returned for revision)*	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
1.1.4	Fracture risk in young and middle-aged adults with type 1 diabetes mellitus: a systematic review and meta-analysis	Published	60%. Systematic search, literature review, data analysis and manuscript drafting	Madhuni Herath: 10% input into study selection and review David R Weber: 5% input into data acquisition and analysis Sanjeeva Ranasinha: 5% input into data analysis Peter R Ebeling: 5% input into manuscript Frances Milat: 5% input into manuscript Helena Teede: 10% input into manuscript	No
1.2.7	Diabetes: a metabolic and reproductive disorder in women	Published	65%. Systematic search, literature review and manuscript drafting	Ethel Codner: 10% input into manuscript JSE Laven: 5% input into manuscript Helena Teede: 20% input into manuscript and critical revisions	No
2.0.3	Recurrent vertebral fractures in a young adult: a closer look at bone health in type 1 diabetes mellitus	Published	60%. Literature review and manuscript drafting	Sarah Catford: 10% input into manuscript Julie Fletcher: 5% input into histomorphometric analysis Phillip Wong: 5% input into manuscript Peter J Fuller: 5% input into manuscript Helena Teede: 5% input into manuscript Frances Milat: 10% input into manuscript	No
2.1.1	Increased prevalence of fracture and hypoglycaemia in young adults with concomitant type 1 diabetes mellitus and coeliac disease	Published	60%. Data collection, analysis and manuscript drafting	Phillip Wong: 10% input into data analysis and manuscript Anouk Dev: 5% input into manuscript Peter R Ebeling: 7.5% input into manuscript Helena Teede: 7.5% input into manuscript	No

				Frances Milat: 10% input into manuscript	
3.1	Obesity, menstrual irregularity and polycystic ovary syndrome in young women with type 1 diabetes: a population-based study	Submitted to *The Lancet Diabetes & Endocrinology*, under review	65%. Data extraction, cleaning, analysis and manuscript drafting	Frances Milat: 10% input into manuscript Anju E Joham: 5% input into manuscript Gita D Mishra: 5% input into manuscript Helena Teede: 15% input into manuscript	No
4.1	Associations between reproductive factors and bone health in women with diabetes: a 15-year longitudinal study	Submitted to *The Journal of Clinical Endocrinology & Metabolism*, under review	65%. Data extraction, cleaning, analysis and manuscript drafting	Frances Milat: 5% input into manuscript Joanne C Enticott: 5% input into data analysis and manuscript Anju E Joham: 5% input into manuscript Gita D Mishra: 5% input into manuscript Peter R Ebeling: 5% input into manuscript Helena Teede: 10% input into manuscript	No

I have / have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: <signature removed> **Date:** 29 Mar 2020

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: <signature removed> **Date:** 29 Mar 2020

ABSTRACT OF THESIS

Type 1 diabetes (T1D) is an autoimmune condition characterised by pancreatic beta-cell destruction, leading to insulin deficiency. The onset of T1D in childhood and adolescence may interfere with development and growth trajectories, with potential deleterious effects on skeletal and reproductive health. Both skeletal fragility and reproductive disorders are emerging, yet under-appreciated complications of T1D, which can contribute to excess morbidity in this cohort. Accumulating evidence within the last decade has confirmed the increased fracture risk, particularly that of hip fractures, amongst individuals with T1D. However, the mechanisms and clinical risk factors contributing to fracture risk in this cohort are not completely understood.

Menstrual and reproductive disorders are common in women with T1D, which can manifest at various stages in the lifespan. The changing landscape of T1D therapies has led to the evolution of menstrual and reproductive disorders in this cohort, where improved glycaemic control has led to the normalisation of menarchal age and improvements in fertility. However, reproductive disorders remain prevalent, with a rise in polycystic ovary syndrome (PCOS), as well as purported earlier menopause. This research aims to evaluate bone and reproductive outcomes in individuals with T1D, to better delineate risk factors and mechanisms, as well as potential interactions between the reproductive and skeletal systems.

This thesis first discusses the determinants of bone health and fracture epidemiology in T1D and knowledge gaps of T1D-related skeletal fragility. I designed and performed a systematic review and meta-analysis to evaluate fracture risk in a younger adult cohort of T1D, without the potential age-related confounders of skeletal fragility, such as menopause and frailty. A salient finding of this paper was the four-fold greater risk of hip fracture in young to middle-

aged adults with T1D, compared to non-diabetic controls. I reviewed the molecular and cellular mechanisms of bone fragility in T1D, with a brief focus on bone microarchitecture deficits. I also performed a cross-sectional study of young adults with T1D attending specialist diabetes clinics at a tertiary institution, identifying concomitant coeliac disease and hypoglycaemia as risk factors for fracture.

I led an extensive review of menstrual and reproductive disorders in T1D with international collaborators, demonstrating insights on mechanisms and identification of knowledge gaps in the literature. The impact of T1D on menstrual and reproductive dysfunction under contemporary management was evaluated from a large observational study of Australian women of reproductive age, where T1D and obesity were found to be independent contributors to menstrual irregularity and PCOS. Lastly, interactions between skeletal and bone health in T1D were examined by way of a longitudinal study of fracture and osteoporosis outcomes in women undergoing the menopause transition. In this study, I showed that women with T1D had earlier menopause, compared to women with type 2 diabetes (T2D) and non-diabetic controls, as well as a higher risk of osteoporosis, suggesting that a shorter reproductive lifespan could mediate skeletal fragility in this cohort.

Overall, the body of work presented in this thesis exemplifies the diverse potential mechanisms contributing to adverse skeletal and reproductive outcomes in individuals with T1D, including the repercussions of contemporary diabetes management, such as hypoglycaemia and weight gain secondary to intensive insulin therapy, on bone and reproductive health, respectively. This work also provides a unique Australian perspective into endocrine complications of women with T1D, by way of two large population-based cohort studies, utilising data extracted from the Australian Longitudinal Study in Women's Health (ALSWH).

ACKNOWLEDGEMENTS

My journey to obtaining a doctoral degree is akin to a passage of self-discovery: initially uncomfortable and frightening, interspersed with uncertainty and a mixture of emotions along the way, culminating in renewed fervour and self-mastery. It has been a rollercoaster ride of triumphs and rejections, albeit one that has enriched me for the better. In these last three years, I learnt a great deal about research, biostatistics and writing manuscripts; but most of all, I gained important life lessons and wonderful friends, colleagues and mentors.

"Do nothing in haste; look well to each step;
and from the beginning think what may be the end."
— Edward Whymper

In writing these acknowledgements, I found it most challenging trying to summarise my thanks and gratitude for the people who have supported me during my doctoral studies – it is not possible to express my true appreciation within the confines of mere paragraphs, but I can only try.

To my supervisors, Professor Helena Teede, Associate Professor Frances Milat and Professor Peter Ebeling, I cannot thank you enough for your unwavering support, guidance and mentorship. You are all exceptional researchers, clinicians and individuals in your own right, and I could not be more humbled to have been under your tutelage. You inspire me to be a fearless woman in Medicine, and to continue to strive for academic and clinical excellence. Thank you for the countless hours you have put in to review my proposals, manuscripts and thesis, your generosity in spirit, teachings and kindness. I could not have done this without you, and my achievements today are yours.

To my colleagues at the Monash Centre for Health Research and Implementation (MCHRI) and the Bone and Muscle Group, you accepted me into the fold with open arms, and I am

thankful for all your help rendered, the constant source of inspiration and encouragement, and most importantly, for the friendships that I know will last beyond my PhD.

To my fellow PhD students at MCHRI, especially Miss Stephanie Pirotta, who has shared a workspace with me for the last three years, thank you for the laughs, commiserations and camaraderie; truly, I could not have asked for more wonderful friends to share this journey with.

To my incredible parents and my brothers, thank you for your unconditional love and for being my perennial cheerleaders. You have always believed in my aspirations, no matter how lofty, yet you keep me grounded and steadfast. Though we are separated by oceans, I keep you all close to my heart.

Last but not least, to my partner-in-life, Raffy, the light of my life and best friend: you have been on this journey with me even before I started. You have supported and reassured me in every step of the way. You have shared in all my joys, seen me at my worst, and continually hold me to the highest standards in everything that I do. You dole out tough love, and bring much-needed perspective and balance to my life. I should also mention my beloved feline Gary, who provided hours of welcome distraction and joy, as I spent most nights typing away.

This research was supported by a Research Training Program Scholarship (formerly known as the Australian Postgraduate Award).

"There is freedom waiting for you,
On the breezes of the sky,
And you ask, "What if I fall?"
Oh, but my darling, what if you fly?"

— Erin Hanson

LIST OF ABBREVIATIONS

ADA – American Diabetes Association

AGEs – advanced glycation end-products

ALSWH – Australian Longitudinal Study in Women's' Health

AMH – anti-Müllerian hormone

BMD – bone mineral density

BMI – body mass index

BMU – basic multicellular unit

CKD – chronic kidney disease

CI – confidence interval

CTX – C-telopeptide

DXA – dual-energy X-ray absorptiometry

EM – early menopause

FN – femoral neck

FRAX® – fracture risk assessment tool

FSH – follicle-stimulating hormone

GnRH – gonadotrophin-releasing hormone

HbA1c – glycated haemoglobin

HH – hypogonadotrophic hypogonadism

HLA – human leukocyte antigen

HPO – hypothalamic-pituitary-ovarian

HR – hazard ratio

HRT – hormone replacement therapy

HRpQCT – high-resolution peripheral quantitative computed tomography

IFG – impaired fasting glycaemia

IGF-1 – insulin-like growth factor 1

IGT – impaired glucose tolerance

IL – interleukin

IOF – International Foundation of Osteoporosis

LADA – latent autoimmune diabetes in adults

LH – luteinizing hormone

LS – lumbar spine

OR – odds ratio

PG – plasma glucose

PCOS – polycystic ovary syndrome

POI – premature ovarian insufficiency

P1NP – procollagen type 1 N propeptide

RANKL - receptor activator of nuclear factor κB ligand

RR – relative risk

SD – standard deviation

SHBG – sex-hormone binding globulin

T1D – type 1 diabetes

T2D – type 2 diabetes

TBS – trabecular bone score

TNF – tumour necrosis factor

WHO – World Health Organization

LIST OF PUBLICATIONS

First-author publications relevant to period of candidature

1. **Thong EP,** Herath M, Weber DR, Ranasinha S, Ebeling PR, Milat F, Teede H. *Fracture risk in young and middle-aged adults with type 1 diabetes mellitus: A systematic review and meta-analysis*. Clin Endocrinol (Oxf). 2018 Sep;89(3):314-323. doi: 10.1111/cen.13761. Epub 2018 Jul 3.
    - Journal impact factor: 2.897; Scientific journal ranking: 1.23; Q1
2. **Thong EP**, Codner E, Laven JSE, Teede H*. Diabetes: a metabolic and reproductive disorder in women.* Lancet Diabetes Endocrinol. 2019 Oct 18. pii: S2213-8587(19)30345-6. doi: 10.1016/S2213-8587(19)30345-6.
    - Journal impact factor: 24.540; Scientific journal ranking: 10.09; Q1
3. **Thong EP**, Catford S, Fletcher J, Wong P, Fuller PJ, Teede H, Milat F*. Recurrent vertebral fractures in a young adult: a closer look at bone health in type 1 diabetes mellitus*. Endocrinol Diabetes Metab Case Rep. 2018 May 10;2018. pii: 18-0010. doi: 10.1530/EDM-18-0010. eCollection 2018.
    - Scientific journal ranking: 0.79, Q2
4. **Thong EP**, Wong P, Dev A, Ebeling PR, Teede HJ, Milat F. *Increased prevalence of fracture and hypoglycaemia in young adults with concomitant type 1 diabetes mellitus and coeliac disease.* Clin Endocrinol (Oxf). 2018 Jan;88(1):37-43. doi: 10.1111/cen.13488. Epub 2017 Oct 23
    - Journal impact factor: 2.897; Scientific journal ranking: 1.23; Q1
5. **Thong EP**, Milat F, Joham AE, Mishra GD, Teede HJ. *Obesity, menstrual irregularity and polycystic ovary syndrome in young women with type 1 diabetes: a population-based study.* [To be submitted]
6. **Thong EP**, Milat F, Enticott JC, Joham AE, Ebeling PR, Mishra GD, Teede HJ. *Associations between reproductive factors and bone health in women with diabetes: a 15-year longitudinal study*. [To be submitted]

Additional peer-reviewed publications during candidature

1. Naderpoor N, Garad R, **Thong EP**, Teede HJ. *PCOS & Diabetes: New Management Guidelines.* Diabetes Management Journal, Nov 2018

LIST OF CONFERENCE PRESENTATIONS

International Conference Presentations

1. Associations between reproductive factors and bone health in women with Type 1 diabetes: a 15-year longitudinal study
    - World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases, Paris, April 2019 (Poster presentation)

2. Contemporary risk of Contemporary risk of menstrual and reproductive dysfunction in women with Type 1 diabetes: a population-based study
    - AEPCOS Update (ENDO Pre-satellite session), New Orleans, USA, March 2019 (Oral presentation)
    - ENDO 2019, New Orleans, USA, March 2019 (Poster presentation) **Presidential Poster Competition Finalist*

3. Trabecular bone score in women with coeliac disease: a cross-sectional study
    a. World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases, Krakow, April 2018 (Poster presentation)

National Conference Presentations

4. Associations between reproductive factors and bone health in women with diabetes: a 15-year longitudinal study.
    a. ANZBMS Annual Scientific Meeting, Darwin, Oct 2019 (Oral presentation) **Roger Melick Young Investigator Award Finalist*

b. Endocrine Society of Australia-Society for Reproductive Biology Annual Scientific Meeting, Sydney, Aug 2019 (Oral presentation) **Bryan Hudson Clinical Endocrinology Award Finalist*

5. Contemporary risk of menstrual and reproductive dysfunction in women with Type 1 diabetes
   a. Endocrine Society of Australia-Society for Reproductive Biology Annual Scientific Meeting, Adelaide, Aug 2018 (Oral presentation) **Awarded Outstanding Clinical Abstract*
6. Increased prevalence of fracture and hypoglycaemia in young adults with concomitant Type 1 diabetes and coeliac disease
   a. Endocrine Society of Australia-Society for Reproductive Biology Annual Scientific Meeting, Perth, Aug 2017 (Poster presentation) **Awarded Best Clinical Research Poster*
7. Increased prevalence of minimal trauma fractures in patients with diabetes: a tertiary centre experience
   a. ANZBMS Annual Scientific Meeting, Brisbane, June 2017 (Poster presentation)
8. Type 1 Diabetes and Bone Health: How can we do better?
   a. Quality Care in Diabetes Award, Melbourne May 2016 (Oral presentation) **Awarded 2nd place*

LIST OF SCHOLARSHIPS, GRANTS AND AWARDS

2020

- Monash University Postgraduate Publication Award ($4924)

2019

- Juvenile Diabetes Research Foundation (JDRF) Travel Grant 2019 ($4000)
- ENDO/Women in Endocrinology Travel Grant 2019 ($1000)
- Australia and New Zealand Bone and Mineral Society (ANZBMS) Travel Grant 2019 ($400)
- Endocrine Society of Australia (ESA) Travel Grant 2019 ($400)
- Presidential Poster Competition Finalist, ENDO 2019, New Orleans
- Bryan Hudson Clinical Endocrinology Award Finalist, ESA Annual Scientific Meeting 2019, Sydney
- Roger Melick Young Investigator Award Finalist, ANZBMS Annual Scientific Meeting, Darwin, 2019

2018

- Outstanding Clinical Abstract Award, ESA Annual Scientific Meeting 2018, Adelaide
- The European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO)-Eli Lilly Travel Scholarship, 2018 (EUR $1000)

2017

- Best Clinical Research Poster ESA Annual Scientific Meeting 2017, Perth
- Research Training Program Scholarship (for duration of 2017– 2020)
- Monash Health Academic Fellowship (for duration of 2017– 2018)

2016

- Quality in Diabetes Care Award Finalist, awarded 2nd place, Melbourne 2016

COURSEWORK AND SHORT COURSES

1. Women in Leadership Course (Monash Partners), Oct 2019
2. Regression Methods in Epidemiology Module, Monash University, completed Jun 2018. (Awarded Distinction)
3. Australian Diabetes Society Insulin Pump and Continuous Glucose Monitoring Workshop, June 2018, Melbourne
4. Good Clinical Practice Training, completed Nov 2017. Awarded Certificate of Completion.
5. Scanco Medical high-resolution peripheral quantitative computed tomography (HR-pQCT) operator training, completed Nov 2017
6. Systematic review course (run by Monash Centre for Health Research and Implementation [MCHRI]/Centre for Clinical Effectiveness [CCE]), completed Oct 2017
7. Introduction to STATA, completed Aug 2017
8. Introductory Biostatistics Module, Monash University, completed Jun 2017. (Awarded High Distinction)
9. Introductory Epidemiology Module, Monash University, completed Jun 2017. (Awarded High Distinction)
10. Australian and New Zealand Bone Mineral Society (ANZBMS) Clinical Densitometry course, completed Mar 2017. Awarded Certification of Completion in Clinical Bone Densitometry.

LEADERSHIP AND EDUCATION ROLES

1. Australian and New Zealand Bone Mineral Society (ANZBMS) Early Career Investigator Committee Member, 2018 – present
    a. Responsibilities include developing a clinical program to engage early career researchers and support professional development
    b. Program organiser for the inaugural 'Clinical Cases in Metabolic Bone Disease Seminar' as part of the ANZBMS ASM
2. Program organiser: ANZBMS and Royal Australasian College of Physicians (RACP) Education Webinar Series, 2019 – present
    a. Application on behalf of the ANZBMS for a competitive grant towards educational webinars for clinicians and trainees
3. Royal Australasian College of Physicians Basic Physician Training Program Supervisor, 2019 – present
    a. Supervision and mentoring of 3 basic physician trainees, overseeing professional and personal development
    b. Provision of educational lectures for junior doctors
4. Working group for development of a mobile phone application for young adults with Type 1 diabetes, Monash Health, 2019 – present
5. Peer reviewer, Clinical Endocrinology (Oxf), 2017 – 2018
6. Clinical Content & Bedside Tutor, Monash University, 2016 – present
    a. Provision of Endocrinology content tutorials for medical undergraduate students
    b. Examiner for third year clinical examinations
7. Monash Health Peer Supporter, 2016 – 2018
    a. Supported junior medical staff with health and wellbeing matters (received formal training as Peer Support Coach)

CHAPTER 1: INTRODUCTION TO ENDOCRINE COMPLICATIONS OF TYPE 1 DIABETES

1.0 TYPE 1 DIABETES

1.0.1 Epidemiology

Type 1 diabetes (T1D) is an autoimmune condition characterised by pancreatic beta-cell destruction, leading to absolute insulin deficiency and lifelong dependence on exogenous insulin. T1D is the major cause of diabetes in youth and accounts for over 85% of diabetes diagnosed in individuals under the age of 20 worldwide, and is therefore commonly known as 'juvenile-onset diabetes'. In Australia, over 60% of new cases of T1D occur in young people under the age of 25 years[1]. The incidence of T1D increases from birth, and peaks in adolescence, between 10 to 14 years of age[2]. Generally, T1D incidence declines after puberty and stabilises in young adulthood, although T1D can present at all ages, even as late as the 9th decade of life[3]. A quarter of T1D cases are diagnosed in adulthood, and this entity of T1D is referred to as 'latent autoimmune diabetes of adults' (LADA), where the progression of pancreatic beta-cell failure is slower than that of juvenile-onset T1D[4].

The International Diabetes Foundation Atlas estimates that there are approximately 500,000 children aged 14 and under living with T1D[5]. The exact number of individuals with T1D worldwide is unknown. In the United States, an estimated 3 million children and adults have T1D. Globally, the incidence of T1D has been increasing by 2 to 5%; however, there appears to be considerable geographic variability in T1D incidence, from as low as 0.1/100,000 per year in China, to as high as 37/100,000 in Finland and Sardinia[2]. In Australia, the incidence of T1D has remained relatively stable in the last decade, at 11 to 13 new cases per 100,000 per year[1]. Genetic factors strongly influence T1D, where the concordance rate is over 60% in monozygotic twins. Individuals with a first-degree relative with T1D have a 1 in 20 lifetime risk of developing the condition, fifteen times higher than that of the general population. The human leukocyte antigen (HLA) complex on chromosome

6, particularly the HLA class II haplotypes, appear to strongly predispose to T1D[6], where 90-95% of young children with T1D carry either one or both susceptibility haplotypes[7]. Additionally, there is a well-established association between T1D and other autoimmune conditions, such as coeliac disease, Addison's disease and autoimmune thyroid disease, owing to genetic similarities of these conditions within the major histocompatibility complex. Although most autoimmune conditions have a female preponderance, T1D appears to affect both males and females equally in childhood. However, populations of European origin, with a high incidence of T1D, appear to have an excess of male cases, whereas a female excess is observed in non-European populations[8].

1.0.2 Diagnosis and management

The diagnosis of T1D is conventionally made based on clinical signs and symptoms suggestive of a catabolic process due to insulin deficiency, such as polyuria, polydipsia, weight loss and marked hyperglycaemia. The initial presentation of T1D in both youth and adults is variable. Children tend to present more acutely with severe symptoms and/or diabetic ketoacidosis (DKA), a severe metabolic complication of T1D characterised by the biochemical triad of hyperglycaemia, ketonaemia and metabolic acidosis, resulting from absolute or relative insulin deficiency in the face of increased counter-regulatory hormones[9]. The onset in adults appears to be more gradual, with beta-cell failure that progresses slowly, often resembling that of type 2 diabetes (T2D).

Prospective longitudinal studies of persons at risk of developing T1D have revealed that T1D is a continuum that progresses sequentially through distinct phases, before the onset of symptoms. T1D develops in three stages (Table 1). Stage 1 is a pre-symptomatic stage defined by the presence of beta-cell autoimmunity with normoglycaemia. Stage 2 is characterised by beta-cell autoimmunity and dysglycaemia, while Stage 3 is when the onset of symptoms begin, with hyperglycaemia and related clinical symptoms. In individuals who

present with classic symptoms of hyperglycaemia and/or DKA, measurement of plasma glucose (PG) is sufficient to diagnose diabetes (random plasma glucose ≥ 11.1 mmol/L). The diagnostic criteria for diabetes is outlined in Table 1, based on the American Diabetes Association (ADA) guidelines[10].

	Stage 1	**Stage 2**	**Stage 3**
Characteristics	• Autoimmunity • Normoglycaemia • Presymptomatic	• Autoimmunity • Dysglycaemia • Presymptomatic	• New-onset hyperglycaemia • Symptomatic
Diagnostic criteria	• ≥2 autoantibodies • No IGT or IFG	• ≥2 autoantibodies • Dysglycaemia: IFG and/or IGT • FPG 5.6 – 6.9 mmol/L • 2h PG 7.8 – 11.0 mmol/L • HbA1c 5.7 – 6.4% or ≥10% increase in HbA1c	• Clinical symptoms • Diabetes by standard criteria
Criteria for the diagnosis of diabetes	FPG ⩾7.0 mmol/L **OR** 2-h PG ⩾11.1 mmol/L during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 1.75 g/kg up to a maximum of 75 g anhydrous glucose dissolved in water. **OR** HbA1C ⩾6.5% (48 mmol/mol) **OR** In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random PG ⩾11.1 mmol/L		

Table 1. Stages and diagnostic criteria for T1D, based on ADA Standards of Medical Care in Diabetes[11]. Definitions are based on venous PG levels. FPG, fasting plasma glucose; IFG, impaired fasting glycaemia; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; HbA1c, glycated haemoglobin

Insulin therapy is essential for survival in T1D, and the goal of insulin therapy aims to mimic the normal physiological insulin secretion patterns. A number of therapeutic options are currently available for individuals with T1D. These comprise intensive insulin regimens such as multiple daily injections of prandial insulin and basal insulin, or continuous subcutaneous insulin infusion (insulin pump therapy)[10]. Other less intensive regimens, such as pre-mixed insulins, have also been used in certain clinical situations. Clinical guidelines for the management of T1D recommend that the majority of individuals with T1D should be treated with intensive insulin therapy, based on established evidence that intensive insulin regimens achieve near-normal glycaemic control and reduce the development and progression of diabetes complications[12,13].

1.0.3 Long-term sequelae and health impacts of T1D

The effect of hyperglycaemia on macrovascular and microvascular complications is well established from longitudinal studies of individuals with T1D. Cardiovascular disease (CVD) is accelerated in T1D and accounts for the leading cause of morbidity and mortality for individuals with diabetes[13]. Data from individuals with T1D attending Australian diabetes centres (mean age 40 years; mean T1D duration of 16 years), demonstrate that cardiovascular risk factors are highly prevalent in this cohort and strongly associated with the development of cardiovascular disease[14]. Coronary artery disease usually develops at a younger age than non-diabetic individuals, particularly if renal disease supervenes[15]. The microvascular complications of diabetes, retinopathy, neuropathy and nephropathy, are the result of structural alterations of capillaries in affected organs, in the setting of longstanding diabetes. While all individuals with diabetes are at risk of developing microvascular complications, those who have had diabetes for a longer duration are more likely to manifest diabetic retinopathy or nephropathy, which are not usually evident until at least after the tenth year of diabetes[16]. The economic burden related to diabetes and related complications are is substantial, with the average annual cost for an individual with T1D being in excess of

$14,000 USD in the United States[17]. In Australia, the total annual cost of diabetes for individuals aged 30 years and above was in excess of $10 billion in 2005, which equates to $15 billion in 2010. Notably, health-related expenditure was substantially higher in those with both micro- and macrovascular complications[18].

While metabolic and vascular-related complications are well-recognised long-term sequelae of T1D, it is now apparent that T1D can also disrupt the reproductive and skeletal systems. The diagnosis of T1D at an early stage of life where growth and development occurs, can alter the growth trajectories in these systems, leading to deleterious consequences later in life. Menstrual disorders, infertility, early menopause and fragility fractures are now increasingly associated with T1D. Furthermore, the impact of contemporary diabetes management, such as weight gain and hypoglycaemia secondary to intensive insulin therapy, can lend itself to adverse reproductive and skeletal outcomes, contributing to the growing morbidity in this cohort.

1.1 SKELETAL FRAGILITY IN TYPE 1 DIABETES

1.1.1 Determinants of bone health

Osteoporosis is a systemic condition in which low bone mass and impairments in bone microstructure leads to increased skeletal fragility, and a consequent increase in fracture risk[19]. Conventionally, the diagnosis of osteoporosis relies on the quantitative assessment of areal bone mineral density (aBMD), measured by dual-energy X-ray absorptiometry (DXA). The World Health Organization (WHO) defines osteoporosis in postmenopausal women and men aged 50 years and older, as having a BMD that lies 2.5 or more standard deviations (SD) below the normal mean for young healthy women (ie. A T-score of ≤ -2.5 SD)[20]. Osteoporosis can also be diagnosed based on clinical criteria with or without BMD, such as in individuals with a prior hip fracture on minimal trauma (defined as a fall from standing

height or equivalent), or in those with osteopenia (T-score between -1 and > -2.5) with a fragility fracture[21]. Low bone mass in children and adolescents is defined as an aBMD greater than 2 SD below the age-adjusted mean value (Z-score < -2 SD). The diagnosis of low bone mass or osteoporosis in young adults, between the age of 20 and 50 years, is less well defined; however, a Z-score of < -2.0 is considered to be below the expected range for age. The Internal Osteoporosis Foundation (IOF) proposes that in young adults with a chronic condition known to affect bone metabolism, a T-score below -2.5 at the spine or hip should be considered diagnostic of osteoporosis[22].

The predominant cause of osteoporosis is due to accelerated bone turnover, associated with oestrogen deficiency in menopause. Osteoporosis that arises from, or is exacerbated by other comorbid conditions or medication exposures, is termed “secondary” osteoporosis[23]. While secondary osteoporosis is most commonly found in premenopausal women and men, as high as 30% of postmenopausal women have been found to have a comorbid condition contributing to bone loss[24]. The causes of secondary osteoporosis are numerous, and include endocrine disorders such as hypogonadism and T1D, gastrointestinal, renal and chronic inflammatory disease, organ transplantation, genetic and metabolic disorders and certain medications[22] (Table 2).

Endocrine • T1D / T2D • Hypogonadism (amenorrhea, anorexia nervosa, Turner syndrome) • Hyperthyroidism • Hyperparathyroidism • Hypercortisolaemia	**Neuromuscular and metabolic** • Cerebral palsy • Epilepsy • Parkinson's disease • Duchenne muscular dystrophy • Glycogen storage disease • Haemochromatosis
Gastrointestinal • Coeliac disease • Inflammatory bowel disease • Chronic liver disease • Malabsorptive syndromes	**Rheumatological** • Rheumatoid arthritis • Connective tissue diseases
Renal • Chronic kidney disease	**Respiratory** • Cystic fibrosis • Chronic obstructive pulmonary disease / asthma
Haematological • Thalassaemia • Leukaemia • Systemic mastocytosis • Multiple myeloma	**Other** • Genetic conditions, eg. osteogenesis imperfecta • Organ transplantation • Vitamin D deficiency
Medications • Glucocorticoids • Antiepileptic drugs • Heparin • Cytotoxic chemotherapy • Cyclosporine • Aromatase inhibitors	**Factors predisposing to falls** • Impaired balance • Obesity • Neuropathy • Medications, eg. sedatives, antihypertensives

Table 2. Causes of and risk factors for osteoporosis and fracture. Adapted from Ferrari and colleagues[22] (*Osteoporosis International* 23:2735–2748, 2012).

While BMD is a major determinant of bone strength, it lacks sensitivity and specificity for fracture risk prediction. Fractures occur in over half of individuals who do not have osteoporosis on BMD criteria, and most women with osteoporosis do not fracture[25]. Therefore, other factors that contribute to skeletal integrity should be taken into account with BMD, when stratifying fracture risk. Bone strength is determined by its material composition and structural design, which comprises geometry, trabeculae distribution, cortical porosity, collagen content and cross-linking[26,27]. Newer imaging modalities, such as high-resolution peripheral quantitative computed tomography (HRpQCT) and trabecular bone score (TBS), allow for volumetric assessments of bone density and geometry, microarchitecture and integrated measurements of bone strength, which provides information about fracture risk that is independent of BMD. Lifestyle factors, such as poor calcium dietary intake, smoking and excess alcohol consumption can contribute to accelerated bone loss. In addition, risks factors for falls, such as frailty, neurological disorders and vision impairment, can also increase the risk for falls, and consequent fracture.

1.1.2 Epidemiology of fractures in T1D

Fragility fractures are associated with chronic pain, disability, and increased mortality. Hip fractures carry a 15–20% increased risk of mortality within 1 year, in addition to a 2.5-fold increased risk of subsequent fracture[28]. Over 9 million osteoporotic fractures occur annually worldwide, placing a considerable load on healthcare and economic systems[29].
The burden of fractures will continue to increase with an ageing population, where in 2025 the number of incident fractures and associated costs are projected to surpass 3 million and $25 billion, respectively, in the United States alone[30]. Under modern therapies, enhanced glycaemic control and reduction in long-term complications have resulted in improved survival for individuals with T1D[31]. Therefore, a growing number of people with T1D will be older, and consequently at risk for osteoporotic fractures[32].

Over the last decade, fragility fractures have gained more recognition as a complication of T1D, as the links between T1D and skeletal fragility become ever more apparent. Evidence from large observational cohort studies have consistently reported a considerably increased risk of fragility fractures in individuals with T1D[33-35], compared with their non-diabetic counterparts. A meta-analysis of femoral neck (FN) and lumbar spine (LS) bone mineral density (BMD) reported modest reductions in BMD at both the FN (-0.055 gm/cm^2, 95% CI -0.065, -0.045) and LS (-0.035 gm/cm^2, 95% CI -0.049, -0.002) regions in individuals with T1D, compared with controls[36]. Indeed, fracture risk is disproportionately elevated in T1D, with only mild reductions in BMD. Data from meta-analyses conducted in 2007 by Vestergaard[37] and Janghorbhani *et al.*[38] demonstrated a 6.94- and 6.3-fold increased risk of hip fracture in individuals with T1D, respectively. A more recent meta-analysis by Shah and colleagues[32] in 2015 reported a lower relative risk estimate of 3.78 for hip fracture in this cohort, which may reflect the availability of more contemporary studies for analysis. Pooled relative risk estimates for any and vertebral (spine) fractures were 3.16 and 2.88, respectively, compared with subjects without diabetes. Stratification of these analyses by fracture type and sex, revealed four- and five-fold increased risks of any and hip fracture in both men and women with T1D, respectively.

The majority of studies on skeletal events in T1D have been conducted in adult cohorts over the age of 40, thus providing little information regarding fracture risk in children and young adults with T1D. In the largest cohort study examining fracture rates in over 30,000 individuals with T1D, Weber and colleagues[33] utilised data from primary healthcare providers throughout the United Kingdom, and reported an increased risk of incident fracture in individuals with T1D, which began in childhood and increased throughout the lifespan. Notably, T1D was associated with a disproportionately increased risk of lower extremity fractures. Overall, there is consistent evidence to suggest that T1D increases the propensity for hip, vertebral and lower limb fractures[32-34,37]. Given the increasing prevalence of T1D and

morbidity and mortality associated with hip fractures, these findings have important public health implications.

1.1.3 Knowledge gaps

Fracture risk is significantly higher in individuals with T1D. However, the potential mechanisms and clinical risk factors leading to skeletal fragility have not been particularly well elucidated. Although BMD is an important determinant of fracture risk, the modest reduction in femoral neck BMD in the T1D cohort does not adequately explain the excessively high risk of hip fracture. Furthermore, estimates of hip fracture risk in T1D from prior meta-analyses are largely derived from studies of older populations, where the intrinsic risk of hip fracture is higher with age. Therefore, the true hip fracture risk in individuals with T1D, without the age-related confounders for bone fragility and falls, is unclear. However, a few cohort studies have reported an elevated hip fracture risk in younger adults. Miao and colleagues reported standardised hospitalisation ratios of 7.6 and 3.4 for hip fracture, in Swedish women and men with T1D aged under 40 years, respectively. Weber and colleagues found that the adjusted hazard ratio of hip fractures in women with T1D in the 30 to 39 year-old age range was 4.16, which surpassed the risk estimates for women with T1D over the age of 60[33].

The fracture risk assessment tool (FRAX®) is a computer-based algorithm that provides estimates of the 10-year probabilities of a major osteoporotic fracture (hip, spine, humerus or wrist) and hip fracture. FRAX® is calibrated according to region-specific epidemiology of death and fracture, and models have since been adapted for 64 countries since its introduction in 2008. FRAX® integrates 10 clinical risk factors into the algorithm, including age, BMI, femoral neck BMD, prior fragility fracture, parental history of hip fracture, current smoking, excessive alcohol intake, long-term use of oral glucocorticoids, rheumatoid arthritis

and/or other causes of secondary osteoporosis. While FRAX® is widely used to provide estimates of fracture risk in the general population, it should be noted that FRAX® underestimates the fracture risk in patients with diabetes[39], although several methods have been proposed to improve the performance of FRAX® in T2D[40]. However, fracture risk stratification in T1D remains challenging, especially in young adults, in whom the use of FRAX® has not been validated[22]. Hence, the evaluation of fracture risk and associated mechanisms in younger adults with T1D is necessary to identify those at highest risk of fracture, and to guide the timing and mode of bone health assessment.

1.1.4 Fracture risk in young and middle-aged adults with type 1 diabetes mellitus: A systematic review and meta-analysis

(*Clinical Endocrinology*)

Received: 25 April 2018 | Revised: 28 May 2018 | Accepted: 5 June 2018
DOI: 10.1111/cen.13761

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ORIGINAL ARTICLE

WILEY

Fracture risk in young and middle-aged adults with type 1 diabetes mellitus: A systematic review and meta-analysis

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Funding information
This study was not funded. EPT is a recipient of the Research Training Program Postgraduate Scholarship and FM is supported by an Endocrine Society of Australia Ken Wynne Post-doctoral Research Award. HT is a National Health and Medical Research Council (NHMRC) Fellow.

Summary

Background: Type 1 diabetes mellitus (T1DM) is associated with skeletal fragility. While previous meta-analyses have demonstrated an increased risk of fracture in individuals with T1DM, little is known about fracture risk in T1DM, in the absence of age-related confounders.

Aims: To determine the risk of fracture in young and middle-aged adults with T1DM aged 18-50 years old.

Design: Systematic review and meta-analysis.

Data sources: Ovid MEDLINE, PubMed, EMBASE, EBM reviews and relevant conference abstracts.

Study inclusion criteria: Studies of adults aged between 18-50 years with type 1 diabetes mellitus, with reported fracture outcomes.

Primary outcomes: Incident or prevalent fracture.

Results: Six studies were included in the meta-analysis. A total of 1724 fractures occurred in 35 925 patients with T1DM and 48 253 fractures occurred in 2 455 016 controls. RR for all fractures was 1.88 (95% CI 1.52-2.32, $P < .001$). Fifty-six hip fractures occurred among 34 707 patients with T1DM and 594 hip fractures occurred in 2 295 177 controls. The RR of hip fractures was 4.40 (95% CI 2.58-7.50, $P < .001$). Females and males with T1DM had a RR of 5.79 (95% CI 3.55-9.44, $P < .001$) and 3.67 (95% CI 2.10-6.41, $P < .001$), respectively.

Conclusions: In the absence of age-related comorbidities, fracture risk remains significantly elevated in young and middle-aged adults with T1DM. Younger age does not mitigate against hip fracture risk in T1DM, and health professionals need to be aware of this risk. Further studies are needed to evaluate the mechanisms of fracture in T1DM.

KEYWORDS
bone metabolism, fracture, hip fracture, type 1 diabetes mellitus, young adults

1 | INTRODUCTION

Both diabetes mellitus and osteoporosis represent important public health concerns associated with rising mortality, morbidity and healthcare costs. Current projections estimate that by 2030, the number of individuals with diabetes will grow to 366 million worldwide. The burgeoning global prevalence of diabetes appears to occur in parallel with the increase in osteoporotic fractures, with a projected 2.6 million hip fractures occurring by 2025.[1] Emerging evidence from large observational studies, particularly in the last

decade, supports a direct detrimental impact of diabetes on skeletal health, particularly in type 1 diabetes mellitus (T1DM).

Type 1 diabetes mellitus is an autoimmune condition characterized by destruction of pancreatic islet cells, leading to absolute insulin deficiency. Several mechanisms have been identified to account for skeletal fragility in T1DM[2] and deficits in bone mineral density (BMD),[3,4] bone geometry,[5] bone microarchitecture[6] and biomechanical properties[7,8] have been identified in humans and animal models of T1DM.[9] Previous meta-analyses[3,10-12] have demonstrated a four- to sevenfold increased risk of hip fracture in T1DM compared to controls; however, significant heterogeneity exists across study cohorts, with one meta-analysis including studies of children[10] and others incorporating predominantly older study cohorts.[3,12] Fractures in children are frequent, compared to young and middle-aged adults; this can largely be attributed to a combination of reduced bone mass and size, physical activity and trauma,[13] rather than true skeletal fragility. Furthermore, peak bone mass is accrued in adolescence[14] and completed by the third decade of life. Hence, fracture risk in children who have yet to achieve maximal growth and bone mass cannot be extrapolated to adults. Conversely, in studies of older adults, potential age-related confounders for bone fragility and falls, such as menopause and sarcopenia, can overestimate the true fracture risk in T1DM.

Type 1 diabetes mellitus disproportionately affects children and young adults, with the peak incidence spanning from birth to 14 years of age.[15] Individuals with juvenile-onset T1DM are consequently exposed to disease for a longer duration throughout their lifetime and may be at risk of developing diabetes-related complications earlier in life. In the largest UK-based prospective cohort study of over 30 000 individuals with T1DM and over 300 000 non-diabetic controls, Weber at al[14] demonstrated that individuals with T1DM had an increased risk of incident fracture across all ages, from birth to age 89 years, as well as a predilection for lower extremity fractures. Similarly, in a population-based study from Scotland, Hothersall et al[16] reported substantially higher hip fracture risks in men and women with T1DM across the ages of 20-49 years, compared to age-matched controls. Thus, the findings from large cohort studies support the notion that young to middle-aged adults with T1DM are equally vulnerable to fracture as their older, postmenopausal counterparts.

While T1DM is recognized as an established risk factor for osteoporosis and fracture, the recommendations for bone health assessment in this cohort is still unclear. Bone health screening programs are commonly targeted at older populations, given the low absolute numbers of osteoporotic fracture in young adults. In the light of mounting evidence for increased skeletal fragility in young adults with T1DM, we undertook a systematic review and meta-analysis dedicated to evaluating fracture risk in a younger adult cohort, with the aims of addressing the following questions:

1. What is the risk of overall fracture in young to middle-aged adults (aged 18-50 years) with T1DM, compared to controls?
2. What is the risk of hip fracture in young to middle-aged adults with T1DM?
3. Is fracture risk in T1DM different between sexes?

2 | METHODS

This systematic review and meta-analysis was performed in accordance with the PRISMA statement.[17] The protocol has been registered with PROSPERO (CRD42017077850) and is available at https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=77850.

2.1 | Search strategy

We conducted a systematic search in several databases including Ovid MEDLINE, PubMed, EMBASE, all EBM reviews from 1980 to present (28 November 2017).

Abstracts from annual scientific meetings of the American Society for Bone and Mineral Research, American Diabetes Association, European Association for the Study of Diabetes, European Calcified Tissue Society and World Congress of Osteoporosis (from 2005 onwards) were also screened.

All aforementioned databases were searched for keywords including:

"Type 1 diabet*" OR "Type I diabet*" OR "T1DM" OR "TIDM" OR "insulin dependent diabet*"

AND "fracture" OR "bone" OR "bone mineral*"

OR "osteoporo*" OR "osteopaeni*" OR "osteopeni*"

The literature search was limited to studies carried out in humans and published in English.

2.2 | Study selection

Study selection was performed by two independent reviewers (EPT and MH). Only studies published in English were screened. Abstracts were assessed if the study fulfilled the inclusion criteria of: (i) cases as adults aged 18 years and above with established type 1 diabetes mellitus and controls as non-diabetic subjects; (ii) criteria for classifying individuals with T1DM was clearly defined; (iii) fracture rates were reported. Studies were excluded if cases also included individuals with type 2 diabetes mellitus, and outcomes were not differentiated by diabetes type, or if no control group was included. Studies comprising post-transplant individuals were also excluded. Fractures occurring at sites other than that considered to be typical of major osteoporotic fractures, such as those of the skull, facial, metacarpals, metatarsals, fingers or toes, were excluded. Full texts of all eligible studies were reviewed and consensus achieved between the two reviewers. Disagreements were resolved with face-to-face discussion or adjudication by the senior author.

2.3 | Data extraction

Information from included studies comprised of: name of first author, publication year, country of origin, study design, study population and recruitment setting, number of participants (cases and controls, male-to-female distribution), mean ages and reported measures of fracture risk, and variables considered in the adjusted fracture risk. If the age range of participants fell outside the 18-50 years age group, we contacted individual authors of eligible studies to obtain secondary data analyses for individuals with type 1 diabetes mellitus and age-matched controls for the prespecified age range.

2.4 | Risk of bias assessment

Methodological quality, as determined by bias analysis, was assessed by two independent reviewers (EPT and MH) using criteria established a priori, outlined in the Monash Centre for Health Research and Implementation (MCHRI) Evidence Synthesis Program critical appraisal template.[18]

Studies were assessed on individual criteria relating to external validity (methodology, inclusion/exclusion criteria, and appropriateness of measured outcomes) and internal validity (selection, attrition, detection and reporting bias and confounders). Studies that fulfilled all, most or few criteria were deemed to have low, moderate and high levels of bias, respectively. Only studies of low-to-moderate bias were included.

2.5 | Statistical methods

Data were analysed using RevMan version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Fracture outcomes were presented as relative risks (RR) with 95% confidence intervals. RRs were calculated from raw data obtained by authors for the specified age ranges, using Stata statistical software: Release 15 (College Station, TX, Statacorp LLC). Heterogeneity was assessed using the I^2 test, where I^2 values greater than 50% and 75% are indicative of moderate and high heterogeneity, respectively. A random-effects model was employed in the analysis if moderate or high heterogeneity between studies were observed. Funnel plots, Begg's adjusted rank correlation test and Egger's regression test of asymmetry were used to assess publication bias. All statistical tests were two-tailed, with a P-value of <.05 considered to be statistically significant. Subgroup analyses were performed by fracture type (hip) and sex.

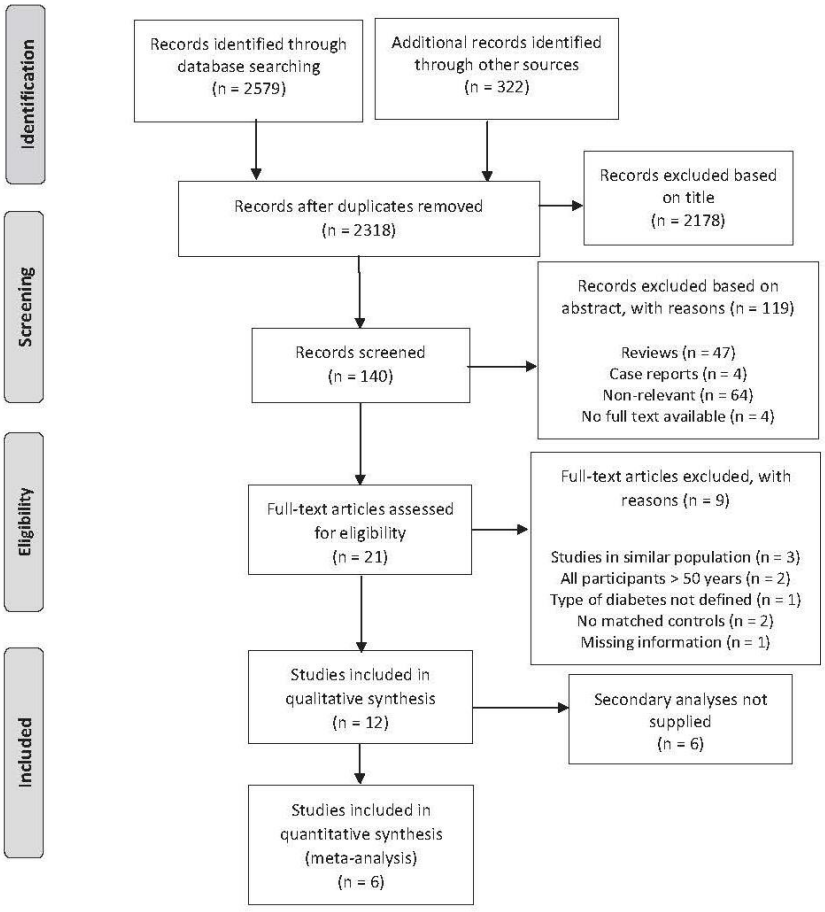

FIGURE 1 Flowchart of study selection. Diagram adapted from Moher et al. (2009)[17]

3 | RESULTS

3.1 | Study selection

After excluding duplicate records, a total of 2901 publications and conference abstracts were identified in the screening search. A total of 2178 articles were excluded based on title, and a further 119 articles excluded based on abstracts. Twelve studies met eligibility criteria to be included in the qualitative analysis, after full-text review of 21 studies. Corresponding authors of individual studies were contacted by electronic-mail to obtain secondary data analyses of fracture outcomes for the prespecified age range. All authors were contacted a second time if a response had not been received after 4 weeks. Of the 12 studies that met criteria, 6 studies were included in the quantitative analysis (Figure 1).

3.2 | Study characteristics

Characteristics of the selected studies are described in Table 1. Of the six studies included, two were cohort studies,[14,16] one was a case-control study[19] and three were cross-sectional studies.[20-22] The two largest studies were cohort studies by Weber et al[14] and Hothersall et al[16] both set in the United Kingdom. The remainder of the studies were conducted in America, Denmark and Belarus. Four studies had fracture events as the primary outcome. Two studies compared BMD as the primary aim, with fracture events being the secondary aim. One study reported solely on vertebral fractures, one reported hip fracture outcomes only, and the rest reported all types of fractures. Fracture ascertainment was determined by vertebral fracture assessment and spinal radiographs in one study,[20] by International Statistical Classification of Diseases and Related Health Problems (ICD) codings or discharge codes in two cohort studies,[14,19] and from hospital admission data in one study.[16] Fractures were self-reported in the two small studies,[21,22] with further confirmation of fracture in Danielson et al.[21] Classification of individuals with T1DM was clearly defined in all studies. Two studies[20,22] were conducted in women only and were included in the analysis despite having participants of a slightly higher age range of participants, up to 55 years, with the justification that only premenopausal or eugonadal women, with no other secondary cause of osteoporosis, were recruited. All studies were population or registry based with the exception of one study, which recruited from a hospital outpatient setting. Overall, the six included studies were heterogenous in terms of study population, classification of T1DM and methodology.

3.3 | Risk of bias across studies

The studies by Vestergaard et al[19], Hothersall et al[16] and Weber et al[14] involved a large population- or registry-based cohort with objective definitions for T1DM and fracture ascertainment and were therefore of higher quality. While Hothersall et al[16] captured hip fractures solely from hospital admission records, the potential for under-reporting of fractures from this study was thought to be low, as almost all hip fractures are managed in hospital. The use of self-reported fracture without further validation in the study by Strotmeyer et al[22] is subject to recall bias, although the majority of clinically important fractures are likely to be memorable. Unlike the other studies, which were population-based, Zhukouskaya et al[20] recruited participants from hospital outpatient clinics, where the selection of patients with potentially more complex or poorly controlled diabetes was thought to account for the fourfold increased odds of vertebral fracture among young adults with T1DM, compared to controls from the community. In addition, the unique methodology of fracture ascertainment in this study, via active screening, led to the detection of asymptomatic vertebral fractures, which would otherwise have been missed clinically. This raises an important consideration of potential under-detection of asymptomatic fractures in the larger registry-linked studies, which relied on clinically detected fractures as outcomes. In the light of significant study heterogeneity, we performed sensitivity analyses, excluding outliers and including only higher quality studies (Table 2).

3.4 | Synthesis of results

A total of 1724 fractures occurred among 35 925 patients with T1DM (4.8%) and 48 253 fractures occurred among 2 455 016 age-matched controls (2.0%). The pooled relative risk (RR) for all fractures in individuals with T1DM was 1.88 (95% CI 1.52-2.32, $P < .001$), compared to controls (Figure 2). The degree of heterogeneity across studies was high ($I^2 = 88\%$, $P < .001$). Fracture outcomes were further stratified by type (hip fracture) and sex, in the prespecified subgroup analyses. Fifty-six hip fractures occurred among 34 707 patients with T1DM (0.16%) and 594 hip fractures occurred among 2 295 177 controls (0.03%). The pooled RR for hip fracture in individuals with hip fracture was 4.40 (95% CI 2.58-7.50, $P < .001$), compared to controls (Figure 3).

Females and males with T1DM had an increased risk of any fracture, compared to their nondiabetic counterparts, with a RR of 1.85 for women with T1DM (95% CI 1.50-2.30, $P < .001$) and a RR of 1.73 for men with T1DM (95% CI 1.37-2.20, $P < .001$). Women and men with T1DM had a near sixfold (RR 5.79, 95% CI 3.55-9.44, $P < .001$) and fourfold (RR 3.67, 95% CI 2.10-6.41, $P < .001$) increased risk of hip fracture, respectively, compared to controls. Statistical analyses were also performed to compare RRs for overall and hip fracture, respectively, between sexes, and no significant differences were found (Table 2).

Sensitivity analyses were performed as previously described and presented in Table 2. The RR for any fracture was 1.53 (95% CI 1.47-1.61, $P < .001$, $I^2 = 0\%$) and 2.25 (95% CI 1.61-3.14, $P < .001$, $I^2 = 94\%$), after the exclusion of outliers and lower quality studies, respectively. There was no funnel plot asymmetry for T1DM and fracture risk. *P*-values of .37 and .85 were obtained for Egger's regression asymmetry test and Begg's adjusted rank correlation test, respectively, indicating a low probability of publication bias.

TABLE 1 Characteristics of studies included for quantitative analysis

First author and year	Type of study	Setting	Country	Study period	Number of participants	Definition of T1DM	Fracture ascertainment
Zhukouskaya et al., 2013[20]	Cross-sectional	Hospital outpatient	Belarus	2007-2011	82 T1DM (26M:56F); 82 controls (22M:60F)	American Diabetes Association criteria	Vertebral fracture assessment and spinal radiographs
Weber et al., 2015[14]	Prospective cohort	Population	UK	1994-2002	Total 30 394 T1DM (17 074M: 13 347F); 303 872 controls (170 421M:133 451F)	Medical diagnoses (read codes) specific to T1DM or diabetes and <35 years old	Diagnosis codes from outpatient electronic medical records consistent with incident fracture, classified by site; surgically-induced fractures or those associated with birth trauma or metastases were excluded
Hothersall et al., 2014[16]	Retrospective cohort study	Population/ registry-based	Scotland	2005-2007	21 033 T1DM/59 585 person-years; controls 3.66million/10 980 599 person-years	Clinical and prescription history (no evidence of lengthy diabetes duration prior to insulin prescription, no co-prescription of oral anti-diabetic drugs except for metformin)	Incident hip fracture admissions in 2005 to 2007 by linkage of diabetes register to national hospital admissions data (ICD codes)
Vestergaard et al., 2009[19]	Case-control	Population	Denmark	2000	4369 T1DM, 484 615 controls	WHO standards	National Hospital Discharge Register & ICD codes
Danielson et al., 2009[21]	Matched, nested cross-sectional	Population	USA	2005	T1DM females 75; controls 75	New cases of T1DM defined as diagnosis age 30, clinical symptoms of hyperglycaemia and insulin-requiring	Self-reported fractures confirmed by physician or radiograph
Strotmeyer et al., 2006[22]	Cross-sectional	Population (volunteer subgroup from prospective study)	USA	Un-known	T1DM females 67; controls 237	Premenopausal women on diabetes registry diagnosed at age <17 years in hospital	Self-reported

BMD, bone mineral density; T1DM, type 1 diabetes mellitus.
[a]Part of a secondary analysis of dataset initially assembled to describe fracture incidence from birth to 89 years, using data from The Health Improvement Network; data obtained from investigators through personal communication.
[b]Part of a secondary analysis of original data set; data obtained from investigators through personal communication.
[c]Part of a secondary analysis of original data set; data obtained from investigators through personal communication.

4 | DISCUSSION

The findings from this meta-analysis demonstrate that young and middle-aged adults are twice as likely to fracture, compared to nondiabetic controls. This is somewhat lower than the previously reported RR of 3.16 in the meta-analysis by Shah et al[10] but can be reasonably expected in a younger cohort of adults. We report a fourfold increased hip fracture risk, which is slightly lower than the RRs of 5.76 and 6.3 reported in previous meta-analyses of predominantly older cohorts by Fan et al[11] and Janghorbani et al[12] respectively. Despite the deliberate exclusion of older and postmenopausal females in our analysis, hip fracture risk in younger adult females with T1DM was almost sixfold higher than controls of similar age. Similarly, younger men with T1DM exhibited a similarly elevated hip fracture risk, nearly four times that of controls. Overall, there was no difference observed in hip fracture risk between sexes.

Increased hip fracture risk in T1DM has been established in previous meta-analyses[3,10,11] and cohort studies.[23-25] However, the majority of such studies have comprised of adults older than 40 years, which could lead to overestimation of fracture risk attributable to T1DM due to confounders such as postmenopausal osteoporosis, increased frailty and susceptibility to falls. Nevertheless, a few cohort studies have demonstrated that hip fracture risk remains elevated in younger adults. A large Swedish prospective registry-based cohort study by Miao et al showed increased

Age (y)	Total fracture events (fractured/total)	Fracture type	Fracture events by gender in those aged between 18-50 years (fractured/total)		OR/RR/HR	Variables adjusted
20-55	20/82 of T1DM vs 5/82 of controls	Vertebral	Males: 6/26 T1DM; 1/22 controls	Females: 14/56 T1DM; 4/60 controls	OR 4.20, 95% CI 1.40-12.70, $P < .01$	Age, sex, BMI, lumbar spine BMD
0-89	2615/30 394 of T1DM vs 18 624/30 3872 of controls	Any fracture	Males: 779/10 874 T1DM; 5367/109 016 controls[a]	Females: 431/8193 T1DM; 2440/82 156 controls[a]	Males: HR 1.55, 95% CI 1.44-1.67, $P < .001$[a]; Females: 1.76 95% CI 1.58-1.96, $P < .001$[a]	Steroid use, prior fracture, CKD
		Hip	Males: 21/10 983 T1DM; 75/109 927 controls[a]	Females: 14/8321 T1DM; 28/82 390 controls[a]	Males: HR 2.55, 95% CI 1.52-4.26, $P < .001$[a]; Females: HR 3.36, 95% CI 1.61-7.10, $P = .001$[a]	
20-84	105/21 033 of T1DM vs 11 733/36 60000 of controls	Hip	Males: 14/8844 of T1DM; 331/1026 350 of controls[b]	Females: 7/6649 of T1DM; 160/107 6510 of controls[b]	Males: RR 5.4, 95% CI 3.5-8.3, $P < .001$[b]; Females: RR 7.9, 95% CI 3.4-18.5, $P < .001$[b]	Age, sex and calendar year
43 ± 27	1703/4369 of T1DM vs 122 952/371 296 of controls	Any fracture	Males: 284/797 of T1DM; 26 201/105 458 of controls[c]	Females: 139/344 of T1DM; 13 677/55 132 of controls[c]	Males: OR 1.67, 95% CI 1.45-1.94, $P < .001$[c]; Females: OR 2.06, 95% CI 1.66-2.55, $P < .001$[c]	Macro- and micro-vascular complications
18-50	Fracture: 28 T1DM, 18 controls	Any	N/A	Females: 28/75 of T1DM; 18/75 of controls	Females: OR 2.3, 95% CI 1.0-5.2, $P < .05$	Not reported
35-55	Fracture: T1DM 33.3% vs controls 22.6%, age-adjusted OR 1.89 (95% CI 1.02-3.49)	Any fracture after age 20 (site not specified)	N/A	Females: 22/67 of T1DM; 54/237 of controls	Females: OR 1.89, 95% CI 1.02-3.49, $P > .05$	Age

standard hospitalization ratios of 7.6 and 3.4 for hip fracture in women and men with T1DM under the age of 40, respectively.[26] In a more recent cohort study, Weber et al[14] reported an adjusted hazard ratio of 4.16 for hip fracture in women with T1DM aged 30-39 years, which was comparably higher than that of their counterparts over the age of 60. While hip fractures are generally uncommon events in young adults, the fourfold risk of hip fracture reported in our meta-analysis raises important clinical concerns about fracture mechanisms in young people with T1DM. With only a modest reduction of 0.055 g/cm^2 in femoral neck BMD in individuals with T1DM reported in a recent meta-analysis by Shah et al,[4] low bone mass alone does not adequately explain the discrepant risk of hip fracture.

Bone geometry and bone microarchitecture are important determinants of bone strength and may provide further insights into the structural mechanisms of hip fracture. Failure to accrue peak bone mass as a result of childhood-onset T1DM may result in smaller and shorter bones in adulthood, which could portend less favourable bone geometry to resist fracture.[27] Only a few studies have evaluated hip structure in adults with T1DM thus far. In a small cross-sectional study of middle-aged males, Miazgowski et al found no difference in hip strength indices between those with T1DM and controls, although there was a nonsignificant trend towards decreased cross-sectional area and moment of inertia in males with T1DM on hip structural analysis (HSA).[28] However, in a more recent study utilizing quantitative computed tomography, Ishikawa et al

TABLE 2 Subgroup and sensitivity analyses

Variable	Studies included (references)	RR, 95% CI, P-value	Heterogeneity (I^2), P-value
Sex and fracture type			
Men			
All fractures	N = 4 (14,16,19,20)	RR 1.73, 95% CI 1.50-2.30, P < .001*	86%, P < .001
Hip fracture	N = 2 (14,16)	RR 3.67, 95% CI 2.10-6.41, P < .001*	58%, P = .12
Women			
All fractures	N = 6 (all studies)	RR 1.85 95% CI 1.37-2.20, P < .001*	71%, P = .004
Hip fracture	N = 2 (14,16)	RR 5.79, 95% CI 3.55-9.44, P < .001*	43%, P = .15
Higher quality studies (all fractures)	N = 3 (14, 16, 19)	RR 2.25, 95% CI 1.61-3.14, P < .001*	94%, P < .001
Excluding outliers[a](all fractures)	N = 4 (14, 19, 21, 22)	RR 1.53, 95% CI 1.47-1.61, P < .001*	0%, P = .85
Ratio of relative risks (RRR) between sexes, by fracture type			
All fractures	N = 6 (all studies)	RRR 0.94, 95% CI 0.68-1.29, Z = −0.41[b]	P = .68
Hip fracture	N = 2 (14,16)	RRR 0.63, 95% CI 0.30-1.33, Z = −1.20[b]	P = .23

*Denotes significant P-value.
[a]Fixed effects model used due to low heterogeneity.
[b]Denotes Z-score (test of interaction).

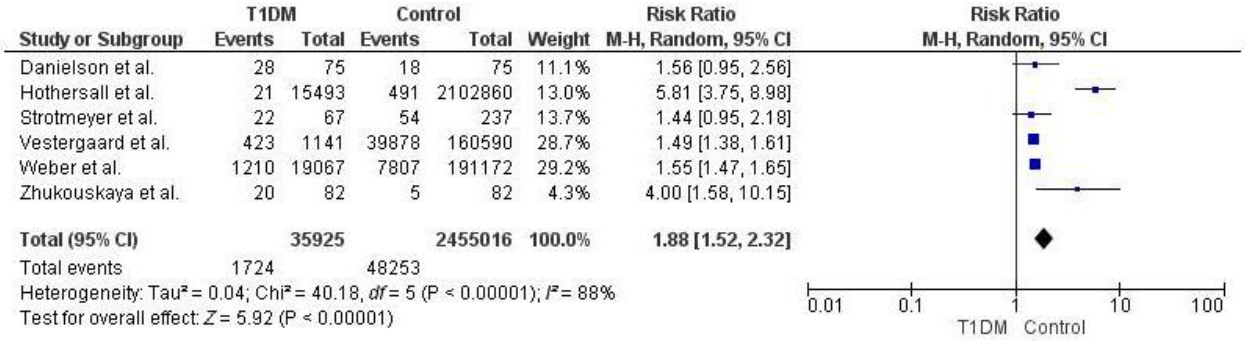

FIGURE 2 All fractures

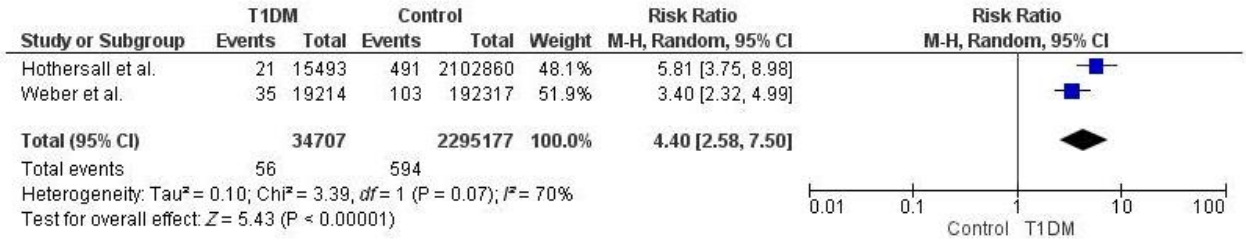

FIGURE 3 Hip fractures

were able to demonstrate significantly reduced cortical volumetric bone mineral density (vBMD) and a higher buckling ratio, a marker of cortical instability, in the intertrochanteric region of young to middle-aged males with T1DM compared to controls.[29] Cortical bone is a key component of bone tissue in the skeleton, and perturbations of cortical bone integrity may predispose individuals to

fracture at sites composed of cortical bone predominantly, such as the femoral neck. In a larger cross-sectional study of middle-aged adult patients with T1DM, Verroken et al found that those with T1DM exhibited cortical bone size deficits at the radial shaft on peripheral quantitative computed tomography (pQCT). These findings were more pronounced at the endosteal envelope, and in association with the finding of lower bone marrow density, are suggestive of a potential role of increased marrow adiposity in the pathogenesis of cortical bone deficits in T1DM.[30]

Microangiopathy may also serve as a mechanism by which T1DM exerts its effects on bone microstructure.[31] Shanbhogue et al[6] described reduced trabecular and cortical vBMD, in addition to thinning of the trabeculae and cortex on high-resolution peripheral quantitative computed tomography (HR-pQCT) in patients with T1DM and microvascular disease, while no differences were observed between individuals with T1DM without complications and nondiabetic controls. As bone is highly vascular, disruption of the microvascular circulation in bone may impair osteoblast function and reduce bone remodelling capacity, thereby leading to decreased bone quality. In addition, it is conceivable that the vascular supply to the femoral head may be compromised in diabetic microangiopathy, which could explain the predilection for hip fracture in T1DM.[32] Vestergaard et al[19] reported a twofold increased risk of fracture in those with T1DM and nephropathy, while Weber et al[14] observed a positive association of retinopathy and neuropathy in individuals with T1DM who had sustained a lower extremity fracture. In light of these findings, it is possible that microangiopathy not only has direct effects on bone quality and may also indirectly potentiate the risk of falls and consequent fracture in susceptible individuals, via visual or physical impediments. Microvascular disease is often regarded as a surrogate of longstanding poor glycaemic control, and the impact of hyperglycaemia on bone health may therefore play an important role in fracture pathophysiology. Previous studies evaluating the effect of hyperglycaemia have yielded inconsistent results, owing to differing methods used in the assessment of glycaemic control. Neumann et al[33] were able to demonstrate a positive association between poor long-term glycaemic control and prevalent fractures in adults with T1DM, independent of BMD. Chronic hyperglycaemia drives the nonenzymatic glycosylation of proteins, leading to the formation of advanced glycation end-products (AGEs), which can disrupt bone collagen matrix and weaken its biomechanical properties.[2] The detrimental effects of AGEs on bone tissue in vivo have been demonstrated by the presence of increased pentosidine, a surrogate marker of AGEs, on bone histomorphometry in individuals with T1DM with prevalent fractures, compared to those without fracture.[34]

Last but not least, hypoglycaemia is a common adverse effect of insulin therapy, and its contribution to fracture risk should not be overlooked. The sympatho-adrenal response to hypoglycaemia is reduced in the face of recurrent hypoglycaemic episodes, and the threshold at which symptoms develop is subsequently lowered.[35] Individuals with hypoglycaemic unawareness are prone to developing severe hypoglycaemia, which can culminate in an altered conscious state predisposing to falls and injury, particularly in the setting of a hypoglycaemic seizure. Several case reports[36-38] have described the traumatic component of such seizures, leading to vertebral fractures. In individuals with underlying bone fragility, avoidance of hypoglycaemia is therefore an important component of fracture prevention.

Overall, there is clear evidence for increased skeletal fragility in T1DM, and our findings support a consistent, but unexplained risk of hip fracture in this younger cohort, designed to exclude age-related confounders. We acknowledge that there are several limitations to this meta-analysis. Firstly, only a small number of studies were included, as procurement of data for participants in our prespecified age range of 18-50 years was limited to authors who had responded. The majority of studies that were excluded in the quantitative analysis were at least a decade old and had small numbers of individuals with T1DM, with the exception of Miao et al[26] which included 25 000 individuals with T1DM. As reported previously, the standardized hospitalisation ratios for hip fracture in a subgroup of adults under the age of 40 were 3.4 and 7.6, for men and women, respectively, which are in keeping with our results. While the potential for reporting bias exists, the inclusion of larger and newer studies may be more representative of contemporary fracture risk in T1DM, in the light of current diabetes therapies. Although there were only two studies included for hip fracture, these were the two largest observational studies, with well-characterized cohorts with objective diabetes and fracture classification. We note that the subgroup analysis of hip fracture risk gave rise to point estimates of relative risks which were much higher than that of the pooled overall fracture risk and its confidence interval. A major limitation of this meta-analysis was the heterogeneity in fracture ascertainment and endpoints of the included studies. Virtually all hip fractures are clinical events, and the detection rate of such fractures is likely to be higher than other types of fracture, which may be clinically silent. It is notable that the pooled relative risk and confidence interval for hip fracture in our analysis is quite similar to the relative risk of 4.0 in the study by Zhukouskaya et al[20] where vertebral fractures were actively screened for in asymptomatic individuals. The study populations included in these analyses originated from America or Europe with predominantly Caucasian participants, thus the findings from this study may not be generalizable to other ethnic groups. The methodologies of the included studies did not allow for the classification of fracture aetiology, and confounders such as trauma-related or sporting injuries in younger adults may over-estimate the risk of fragility fractures in this cohort. Data on body mass index, glycaemic control, duration of diabetes, presence of microvascular complications and concomitant autoimmune disease were not available for all included studies.

To our knowledge, this is the first meta-analysis of fracture risk specifically targeted at young to middle-aged adults with T1DM, with application of strict selection criteria to reduce heterogeneity and confounding due to age-related factors. We included the two largest cohort studies to date,[14,16] comprising

over 34 000 individuals with T1DM, and our findings for hip fracture risk are consistent with previous meta-analyses and cohort studies.

5 | CONCLUSIONS

In a young to middle-aged cohort of type 1 diabetes mellitus (T1DM), we show that hip fracture risk is comparable to previously reported relative risks (RRs) of between 4 and 6, albeit in predominantly older adult study populations.[3,11,12] Young adults with T1DM may be at risk of fracture at a younger age compared to their nondiabetic counterparts. The impact of a hip fracture in a young adult is potentially devastating, with ramifications of physical and psychological morbidity, in addition to increased mortality. Although T1DM is widely acknowledged as a risk factor for secondary osteoporosis and fracture, there is no consensus on the timing and modality of bone health assessment in this cohort. However, we show that young adults with T1DM have an increased risk of fracture, and awareness is needed early in diagnosis. While the absolute numbers for hip fracture are low in this younger cohort, we urge clinicians to be cognizant of the diverse risk factors for skeletal fragility, and treat reversible causes where possible. Individuals with poorly controlled T1DM and microvascular complications appear to be most at risk of fracture, based on observational studies. Concomitant comorbidities that may contribute to fracture risk, such as hypogonadism, autoimmune conditions and hypoglycaemia, should be identified and managed. Imaging modalities that allow indirect assessment of bone microarchitecture and structural properties, such as trabecular bone score, HR-pQCT and hip structural analysis, may be a useful adjunct to DXA in this cohort, where bone mineral density (BMD) is but one of many contributors to fracture risk. Further research evaluating hip fracture mechanisms in T1DM, screening and assessment, is needed to guide practice.

ACKNOWLEDGEMENTS

The authors acknowledge and thank the following individuals for their help with provision of data analyses: Shona Livingston, Helen Colhoun and the Scottish Care Information-Diabetes Collaboration database; David R. Weber, Michelle Denburg and The Health Improvement Network database, UK; Peter Vestergaard, the Danish Hospital Discharge Register and Psychiatric Central Register.

CONFLICT OF INTEREST

Nothing to declare.

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How to cite this article: Thong EP, Herath M, Weber DR, et al. Fracture risk in young and middle-aged adults with type 1 diabetes mellitus: A systematic review and meta-analysis. *Clin Endocrinol (Oxf)*. 2018;89:314–323. https://doi.org/10.1111/cen.13761

1.2 Female Reproductive Health Across the Lifespan in Type 1 diabetes

1.2.1 The hypothalamic-pituitary-ovarian axis

Reproductive dysfunction in T1D results from perturbations at different levels of the gonadotropic axis, including the hypothalamus, pituitary and ovary. These perturbations arise from the combined effects of insulin deficiency and uncontrolled hyperglycaemia that disrupt the physiological functioning of various metabolic signals involved in the regulation of the reproductive system[41]. The hypothalamic-pituitary-ovarian (HPO) axis is a tightly regulated circuit that controls female reproduction (Figure 1). Gonadotropin-releasing hormone (GnRH) is released in a pulsatile fashion from the hypothalamus at the onset of puberty, which dictates the release of pituitary gonadotrophins, luteinizing hormone (LH) and follicular stimulating hormone (FSH) from the anterior pituitary. LH and FSH bind to ovarian receptors and signal the release of female reproductive hormones, oestrogen and progesterone. Oestrogen and progesterone are released in changeable concentrations throughout the menstrual cycle, resulting in the follicular (low oestrogen) phase and the luteal (high oestrogen) phase. These phases are separated by ovulation, and end with either fertilization or menstruation, in a eumenorrheic woman[42].

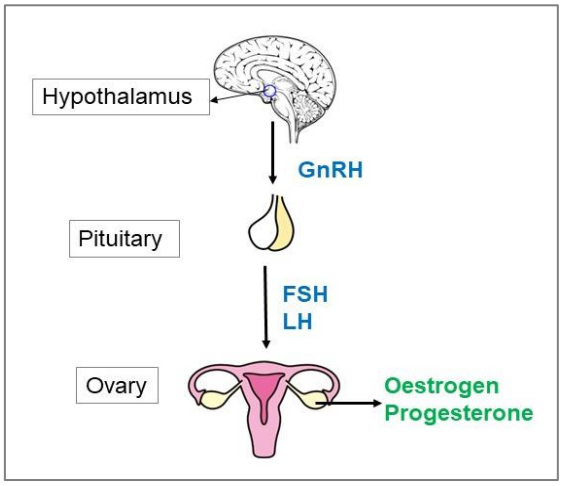

Figure 1. The hypothalamic-pituitary-ovarian axis. (Adapted from Livshits & Seidman[43])

Hypogonadotrophic hypogonadism (HH) is a clinical syndrome that arises from gonadal failure, due to absent or inadequate hypothalamic GnRH secretion or failure of pituitary gonadotrophin secretion[44]. HH is common in insulin deficiency, as insulin is a key regulator of the HPO axis[45]. Insulin stimulates GnRH release, leading to downstream secretion of pituitary gonadotrophins, LH and FSH, and ovarian sex steroid production. In the case of T1D, animal models with severe insulin deficiency exhibit hyperglycaemia and a negative-energy state. Leptin, a hormone secreted by adipocytes, is responsible for energy homeostasis regulation, is consequently reduced in this setting. Both insulin and leptin deficiency inhibit expression of kisspeptin, a neuropeptide which is critical for the maturation and function of the reproductive axis[46]. In murine models of T1D, administration of kisspeptin was able to reverse hypogonadism associated with insulin deficiency[47]. While animal studies have demonstrated the roles of insulin and leptin in the regulation of the HPO axis, hyperglycaemia has also been postulated to have an inhibitory effect on hypothalamic GnRH secretion. Therefore, the pathophysiology of HH in T1D is likely due to the direct effect of insulin deficiency, as well as the indirect effects of hyperglycaemia and severe metabolic disruption.

1.2.2 Menarche and menstrual disorders

Menarche is the first occurrence of menstruation and signifies the beginning of a woman's reproductive life. Menarche occurs between age 10 to 16 years on average in girls in developed countries. Determinants of menarchal age include, but are not limited to, genetics, socioeconomics, general health, exercise and nutritional status. The average age at menarche has fallen in the last century, from 16.5 years in 1840 to 13 years in the 20th century, and may be attributable to improvements in health and environmental conditions[48].

Prior to the invention of insulin therapy, individuals with T1D exhibited a catabolic state, with weight loss and failure to thrive. Pubertal and menarchal delay, amenorrhea and oligomenorrhea were common, attributable to HH in the setting of poor metabolic control. The effect of uncontrolled hyperglycaemia on reproductive dysfunction is two-fold – firstly, by contributing to HH; and secondly, through exerting toxic effects on ovarian follicles. Sustained hyperglycaemia can lead to ovarian toxicity via the deposition of advanced glycation end-products (AGEs)[49]. AGEs are a complex and heterogenous group of compounds that arise from the non-enzymatic glycosylation of proteins and cell components as a result of chronic hyperglycaemia, and can contribute to the development of microvascular complications in diabetes by accumulating in the circulation and various tissues. The advent of insulin therapy in 1923 led to subsequent improvements in pubertal development and menstrual regularity, but did not completely abolish these problems.

In 1993, the landmark Diabetes Control and Complications Trial (DCCT) demonstrated that strict glycaemic control was able to reduce long-term cardiovascular and microvascular complications of T1D, compared with conventional therapy, which aimed to maintain safe asymptomatic glucose control[13]. Intensive insulin therapy, consisting of three or more insulin injections a day or insulin pump therapy, has since been adopted widely as contemporary management of T1D. Prior to intensive insulin therapy, menarche in girls with T1D were delayed by an average of 12 months, compared with their non-diabetic peers. In the 21st century, the age of menarche in girls with T1D appear to be similar to that of their peers, although oligomenorrhea and increased cycle length remain prevalent in adolescents and young women with T1D[45,50-53]. Menarchal delay and oligomenorrhea have been linked to a pre-pubertal onset of T1D, longer duration of diabetes and poor glycaemic control[54-56], respectively. Menstrual disturbance remains common in adolescents with T1D, where contemporary studies have reported a 30% prevalence of oligomenorrhea, defined as having menstrual cycles greater than 36 days[51,56]. Menstrual cycle abnormalities in women with T1D are less well studied, although the frequency of oligomenorrhea is around 20 to 40%, with

16% reporting amenorrhea[52,53]. Poorer glycaemic control is associated with oligomenorrhea, where a 1% increase in HbA1c increases the risk for oligomenorrhea by 5-fold[56]. Concomitant autoimmune conditions, such as thyroid and coeliac disease, may also account for the increased prevalence of menstrual disorders in T1D. Untreated hyperthyroidism and coeliac sprue are recognised causes of secondary amenorrhea, oligomenorrhea and anovulation[57,58], and should be excluded in women with T1D presenting with menstrual dysfunction.

1.2.3 Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age, where up to one in six women of reproductive age are affected. This is a heterogeneous condition, underpinned by insulin resistance, dysglycaemia, overweight and obesity. The repercussions of PCOS are far-reaching, involving reproductive, metabolic and psychological impacts[59]. The diagnosis of PCOS is based on clinical and/or biochemical hyperandrogenism, ovarian dysfunction (oligoanovulation and/or polycystic ovarian morphology), and exclusion of other aetiologies for androgen excess related conditions[59].

Traditionally associated with T2D, overweight and obesity, PCOS is now increasingly more common in young women with T1D. The pooled prevalence of PCOS in young women with T1D was 24% in a meta-analysis[60], which surpasses the prevalence estimates in the general population. Hyperandrogenism, ovulatory and menstrual dysfunction can occur in the setting of exogenous insulin therapy. Subcutaneous administration of insulin bypasses the ‘first pass’ metabolism in the hepatic portal circulation, leading to a high concentration of insulin in the circulation[61,62]. Insulin acts on its own receptor in the ovary to stimulate follicle growth and increase ovarian androgen production, and augments adrenal sex hormone production and pituitary LH release, leading to biochemical hyperandrogenism. The biochemical profile and clinical presentation of PCOS in women with T1D may be different from women with

PCOS alone. Sex-hormone binding globulin (SHBG) levels are often increased or normal, due to low hepatic insulin concentrations, so that free androgen levels are lower than in women with PCOS without T1D, where SHBG is usually reduced[45]. While screening for PCOS is recommended in women with T2D, there is little guidance whether PCOS should also be screened for in T1D. Given the established impact on reproductive dysfunction and adverse metabolic outcomes, PCOS should be considered in women with T1D.

1.2.4 Fertility

Fertility is the natural potential to produce offspring, and is measured by live offspring born per couple, whereas fecundity relates to potential for reproduction. Only a handful of studies have investigated fertility in women with T1D to date, with consistent findings of reduced fertility in this cohort. An international study of individuals with T1D observed that those with T1D had significantly fewer offspring, compared with their non-diabetic siblings. This effect appeared to be more pronounced in women and those with childhood-onset disease[63]. A Finnish cohort study also found that both men and women with T1D had fewer livebirths than matched controls[64]. A Swedish cohort study of women hospitalised for T1D between 1965 and 2004 reported that the standard fertility ratio (SFR, ratio of observed to expected number of livebirths) was reduced by 20% in women with T1D, particularly in those diagnosed prior to 1985 and in women with microvascular and cardiovascular complications[65]. It is not clear whether poor metabolic control directly affects fertility; however, improved glycaemic control under contemporary therapy may improve fertility outcomes in women with T1D. On the other hand, there is speculation that exogenous insulin and hyperinsulinaemia could lead to increased follicle recruitment and subsequent accelerated depletion of ovarian reserves[49]. A more recent Taiwanese population-based cohort study reported a 33% reduction in live birth rates in women with T1D, which was even lower when combined with hyperthyroidism[66]. Reduced fertility may also be the result of anovulation from PCOS, although the literature examining these associations in T1D is

lacking. However, a recent secondary analysis of reproductive-aged female participants of the DCCT suggests that the risk of infertility appears to be increased, even after adjusting for menstrual irregularity[67].

1.2.5 Menopause

Menopause is the natural cessation of a woman's menstrual cycle, which marks the end of female reproduction. This may occur spontaneously (natural menopause) or secondary to an iatrogenic cause (secondary menopause). The median age at natural menopause derived from epidemiological studies, is 48 to 52 years among women from developed nations[68]. Similar to menarche, there is marked variability in age at natural menopause, which is influenced by ethnic and genetic factors, environmental exposures and stress throughout the life[69,70]. The timing of the final menstrual period may be a marker of aging and general health, which has important clinical implications. Later age of menopause has been associated with longer overall survival and reduced all-cause mortality, reduced risks of cardiovascular disease, osteoporosis and fracture, albeit higher risks of breast, endometrial and ovarian cancers[70]. Menopause or loss of ovarian function that occurs earlier than the age of 40 and 45 years, are termed premature ovarian insufficiency (POI) and early menopause (EM) respectively. POI and EM can occur spontaneously, or arise as a result chemotherapy, radiotherapy or surgery. Both POI and EM have been associated with deleterious consequences on cognition, mood, cardiovascular, bone and sexual health[71].

There is limited information regarding the reproductive lifespan and menopause in women with T1D, although data from large observational cohort studies have suggested that menopause may be earlier in T1D, compared with women without diabetes. A tendency for later menarche and earlier menopause was observed in women with T1D, compared with their non-diabetic sisters and unrelated healthy controls, ultimately shortening their reproductive lifespan by 6 years[72]. A large European longitudinal study reported an

association of earlier menopause in women diagnosed with diabetes before the age of 20, compared with controls, which was independent of age, smoking and other reproductive factors[73]. Several theories for this phenomenon have been postulated. Firstly, the process of ovarian ageing may be accelerated by chronic metabolic and vascular dysfunction. The presence of diabetes-related sequelae, including proliferative retinopathy and nephropathy, could be a surrogate marker for microvascular damage in the ovary[49,72]. Secondly, concomitant autoimmune disease is common in T1D, and women with T1D are at increased risk of autoimmune oophoritis, particularly amongst those who also have circulating adrenal antibodies[74]. Last but not least, insulin has a stimulatory effect on ovarian granulosa cells to increase follicle recruitment at each menstrual cycle[55]. It is thought that the exogenous hyperinsulinaemia resulting from a non-physiological mode of insulin absorption could lead to depletion of ovarian follicular stores at a faster rate, ultimately leading to earlier menopause. Lower concentrations of anti-Müllerian hormone (AMH), a biomarker of ovarian reserve, has been reported in women with T1D in the fourth decade of life, which could signify premature ovarian ageing[75,76].

1.2.6 Diabetes: a metabolic and reproductive disorder in women

(*The Lancet Diabetes & Endocrinology*)

Review

Diabetes: a metabolic and reproductive disorder in women

Eleanor P Thong, Ethel Codner, Joop S E Laven, Helena Teede

Reproductive dysfunction is a common but little studied complication of diabetes. The spectrum of reproductive health problems in diabetes is broad, and encompasses delayed puberty and menarche, menstrual cycle abnormalities, subfertility, adverse pregnancy outcomes, and potentially early menopause. Depending on the age at diagnosis of diabetes, reproductive problems can manifest early on in puberty, emerge later when fertility is desired, or occur during the climacteric period. Historically, women with type 1 diabetes have frequently had amenorrhoea and infertility, due to central hypogonadism. With the intensification of insulin therapy and improved metabolic control, these problems have declined, but do persist. Additional reproductive implications of contemporary diabetes management are now emerging, including polycystic ovary syndrome and hyperandrogenism, which are underpinned by insulin action on the ovary. The sharp rise in type 2 diabetes incidence in youth suggests that more women of reproductive age will encounter diabetes-related reproductive problems in their lifetimes. With an ever increasing number of young women living with diabetes, clinicians need to be aware of and equipped for the challenges of navigating reproductive health concerns across the lifespan.

Lancet Diabetes Endocrinol 2019

Published Online
October 18, 2019
https://doi.org/10.1016/S2213-8587(19)30345-6

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Introduction

Type 1 and type 2 diabetes are common conditions with increasing global prevalence. Whereas the incidence of type 1 diabetes is growing slowly, there has been a staggering rise in the number of adolescents and young adults diagnosed with type 2 diabetes with substantial implications for disease burden and health-care costs.[1,2] In the USA alone, diabetes affects nearly 5 million people aged between 18 and 44 years, representing 4% of individuals in this age group.[3] As the incidence of type 1 and type 2 diabetes continues to increase in young women, clinicians will face the rising challenge of managing reproductive concerns alongside diabetes care.

Reproductive dysfunction is common in women with type 1 diabetes, up to 40% of whom will have substantial menstrual or reproductive disorders throughout their lifetime.[4] With the onset of type 1 diabetes occurring in childhood and adolescence, reproductive abnormalities can surface early in the reproductive lifespan, manifesting as pubertal delay and primary amenorrhoea. Additionally, menstrual cycle disturbance, subfertility, well known pregnancy complications, and earlier menopause have been associated with type 1 diabetes.[5–7] The intensification of insulin therapy during the past two decades appears to have ameliorated some of these problems,[4] but the reproductive health of women with type 1 diabetes undergoing contemporary management is unclear. Furthermore, new reproductive implications are emerging, including polycystic ovary syndrome and hyperandrogenic traits in young women with type 1 diabetes (figure 1).[8,9]

Previously a condition associated with ageing, type 2 diabetes incidence is increasing in a younger demographic with adverse lifestyles and inter-related insulin resistance, polycystic ovary syndrome, and obesity. The earlier onset of type 2 diabetes in young women, particularly in the second and third decades of life, overlaps with the reproductive life stage, with potential fertility and pregnancy implications. Despite having a comparatively shorter duration of diabetes than their counterparts with type 1 diabetes, women with type 2 diabetes appear similarly at risk of reproductive disorders, although the literature in this area is scarce. The mechanisms of reproductive dysfunction in type 2 diabetes could be largely attributable to concomitant obesity, polycystic ovary syndrome, and endogenous and exogenous hyperinsulinaemia.

In the context of the evolving prevalence, treatments, and associated disease patterns of type 1 and type 2 diabetes, we aim to examine the effect of diabetes on female reproductive function across life stages. Type 1 and type 2 diabetes are common yet clinically distinct conditions with diverse reproductive manifestations, and we will therefore discuss each of their clinical implications separately when relevant.

Menstrual dysfunction

Menstrual disorders in type 1 diabetes

Menstrual abnormalities are far more common in women with type 1 diabetes than in non-diabetic women.[4] Indeed, before the introduction of insulin therapy in 1923, primary and secondary amenorrhoea, infertility, and the absence of pubertal development were common in women with type 1 diabetes.[10] Although type 1 diabetes has been associated historically with delayed puberty, contemporary studies report that the onset of puberty in type 1 diabetes appears similar to that of their peers.[11,12] Codner and colleagues[11] showed that the secular trend towards a younger age of onset of puberty observed in the healthy population is also observed in adolescents with type 1 diabetes, although thelarche (onset of pubertal breast development) appears still to be delayed.[13] Menarche occurs later during pubertal development, and a mild delay in menarche in type 1 diabetes is still observed in some parts of the world.[14–17] Another study showed that a later age of menarche in type 1 diabetes was associated with a doubled risk of developing diabetic nephropathy and retinopathy[18]. In addition, an inverse relationship was observed between age at onset of diabetes and age of menarche, which is probably related to the general impact of poor metabolic control.

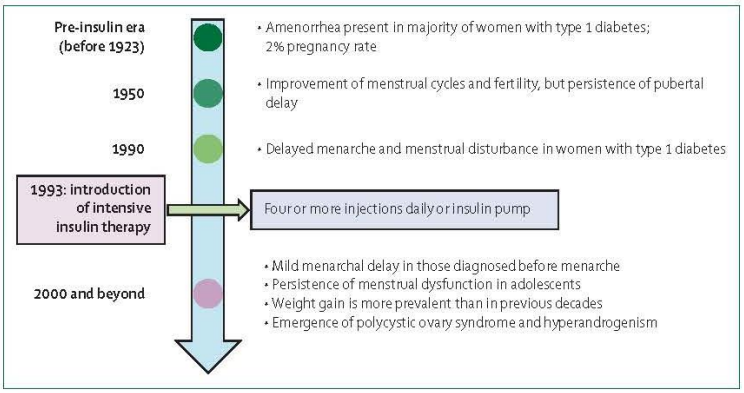

Figure 1: Evolution of menstrual disorders in women with type 1 diabetes

Although menstrual disorders have declined following the introduction of insulin and the subsequent intensification of therapy, these abnormalities remain prevalent (figure 1). Oligomenorrhoea and increased cycle length are the most prevalent menstrual cycle abnormalities observed in contemporary type 1 diabetes groups. Menstrual dysfunction is especially prevalent in adolescents with type 1 diabetes, reported at 20–80%,[17,19,20] compared with 20–40% in adult women with type 1 diabetes.[21–23] A prospective study evaluating menstrual cycles in adolescents with and without type 1 diabetes showed that the risk of menstrual irregularities was six times greater in women with type 1 diabetes than in women without, and the risk became even greater with increasing HbA_{1c}.[24] Gaete and colleagues[25] showed that the menstrual cycle length was 5 days longer for each point of increase in HbA_{1c}, emphasising the relationship between metabolic and reproductive dysfunction in type 1 diabetes.[19,24–26]

Menstrual disorders in type 2 diabetes

Although women who have early menarche have a heightened risk of developing type 2 diabetes later in life, there are no studies on the timing of menarche among adolescents with type 2 diabetes, because of the exceedingly low prevalence of type 2 diabetes in prepubescent girls. The prevalence of oligomenorrhoea is reportedly higher in women with type 2 diabetes than in women without.[27,28] In a multicentre study of 190 adolescent girls with recently diagnosed type 2 diabetes, 21% had irregular menses, which was associated with elevated BMI and alterations in sex steroid and sex hormone-binding globulin (SHBG) concentrations.[29] Indeed, in type 2 diabetes, the interaction between obesity, insulin resistance, and reproductive function becomes more relevant, with increased BMI closely related to ovulatory dysfunction[30] and menstrual cycle abnormalities, independent of diabetes. The distribution of adiposity also affects menstrual disorders, for which an increased waist-to-hip ratio appears to portend a higher risk of oligomenorrhoea in women aged 20–40 years.[31] A further interacting factor is that polycystic ovary syndrome, a common condition with a high prevalence of early onset type 2 diabetes,[32] is also underpinned by insulin resistance and obesity, with associated reproductive abnormalities, as discussed later in this Review. More research is needed to clarify the interactions between polycystic ovary syndrome, type 2 diabetes, and obesity. It remains unclear whether patients with type 2 diabetes without polycystic ovary syndrome or excess body weight[33–35] or with other forms of diabetes, such as maturity onset diabetes of the young, have menstrual irregularities.

Mechanisms of interactions between diabetes and reproductive function

Diabetes and hypothalamic–pituitary function in type 1 diabetes

As early as 1925, Joslin described the important impact of insulin therapy on reproductive function in women with diabetes.[36] In women with type 1 diabetes who had pubertal delay and primary and secondary amenorrhoea, insulin therapy led to improvements in pubertal development and menstrual regularity. In the 1980s, hypogonadotropic hypogonadism was described in patients with type 1 diabetes and poor metabolic control, with decreased concentrations of luteinising hormone (LH), follicle-stimulating hormone (FSH), and oestradiol.[37–39]

Hypogonadotropic hypogonadism is reasonably common in absolute insulin deficiency, as insulin is an important regulator of the hypothalamic–pituitary–gonadal axis (figure 2). Cell lines and cultures derived from hypothalamic cells show that insulin stimulates gonadotropin-releasing hormone (GnRH) secretion.[40] Knockout mice with a specific deletion of the brain insulin receptor have hypogonadotropic hypogonadism and infertility.[41] These animals respond to exogenous administration of GnRH by increasing LH concentrations, which suggests that an absence of insulin action decreases pituitary GnRH stimuli.

Studies in streptozotocin-treated mice, an animal model with severe insulin deficiency, have deepened understanding of the mechanism leading to hypogonadism in uncontrolled diabetes. These mice have uncontrolled hyperglycaemia, leading to a catabolic state with decreased fat mass and diminished serum leptin concentrations. Insulin and leptin deficiency inhibit central nervous system expression of kisspeptin, an important stimulus of GnRH.[42] Exogenous administration of kisspeptin has been shown to reverse hypogonadism in the absence of insulin.[43] Animal models have thus helped to identify the role of peripheral signals, such as insulin and leptin, on the regulation of the hypothalamic–pituitary–gonadal axis.[4] Less well studied mechanisms postulated to play a role in the pathophysiology of hypogonadism in women with type 1 diabetes include the inhibitory effect of hyperglycaemia on the hypothalamic secretion of GnRH[44,45] (figure 2). Changes in central

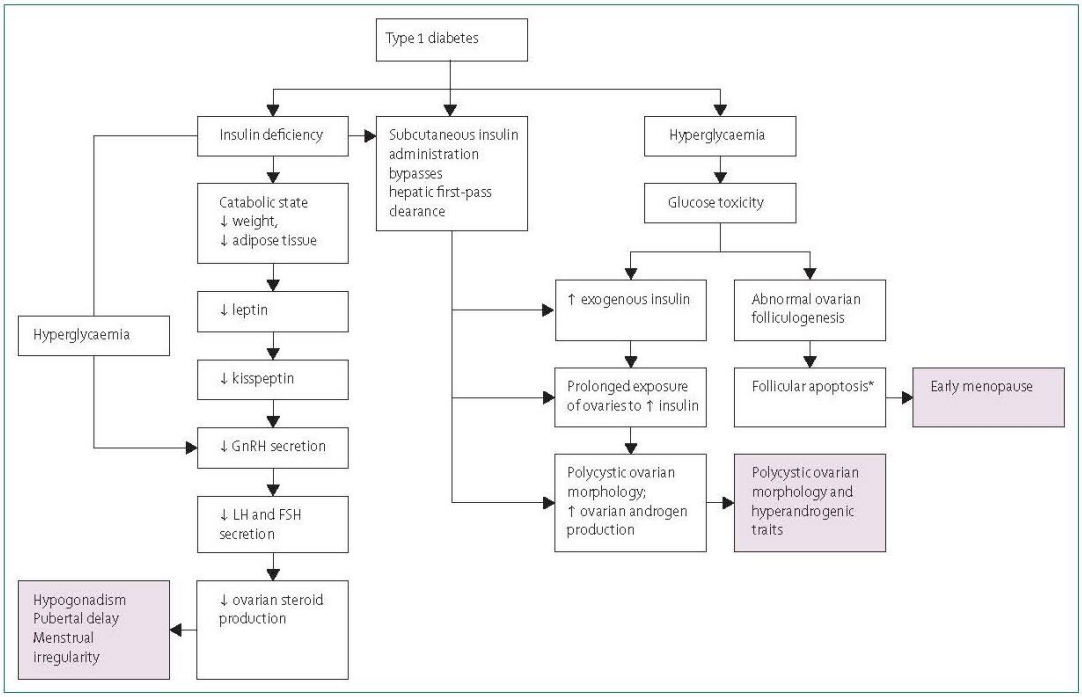

Figure 2: **Mechanisms of interactions between type 1 diabetes and reproductive function**
GnRH=gonadotropin-releasing hormone. LH=luteinising hormone. FSH=follicle-stimulating hormone. Reproduced from Codner and colleagues.[4] *Shown in animal studies.

nervous system mediators other than kisspeptin might also have a role in the diminished GnRH secretion.[4] Regardless of the mechanism, poor metabolic control appears to have deleterious effects on reproductive function.

Secondary hypogonadism might play a role in the pathophysiology of complications in women with type 1 diabetes through oestrogen deficiency.[46] Cardiovascular disease and osteopenia occur at an unusually early age in type 1 diabetes, at a time when healthy, premenopausal women are protected against these conditions.[47] Oestrogen deficiency from secondary hypogonadism could underpin the adverse metabolic and skeletal outcomes in this cohort. Optimal glycaemic control in type 1 diabetes is therefore needed to reduce the risk of long-term complications both directly and indirectly (via reproductive function).

Diabetes, insulin, and ovarian pathology

Insulin, insulin resistance, and hyperglycaemia[4,48] might all contribute to ovarian dysfunction in both type 1 and type 2 diabetes. The ovary expresses insulin receptors in the granulosa, theca, and stromal compartments. Insulin binds to these receptors, and ovarian insulin-like growth factor 1 (IGF-1) receptors,[49] mimicking FSH and LH ovarian stimulation in an effect known as co-gonadotropin. In vitro studies have shown that insulin can stimulate these receptors to increase androgen, oestrogen, and progesterone production by granulosa and theca cells.[50–52] Serum insulin and testosterone concentrations are positively correlated, whereas insulin-sensitising treatments decrease androgen concentrations.[53,54]

Type 1 diabetes and the ovary

Studies in prepubescent girls with type 1 diabetes have shown elevated anti-Müllerian hormone (AMH) concentrations, which suggests an increased number of small growing follicles, indicating a stimulatory effect of insulin therapy on granulosa cells.[55] Physiologically, the pancreas secretes insulin to the portal vein, with substantial first-pass clearance.[56,57] In type 1 diabetes, subcutaneous insulin is absorbed directly into the systemic circulatory system, bypassing first-pass hepatic clearance and thus exposing the ovary to increased insulin concentrations. Chronic exposure to high systemic concentrations of insulin stimulates ovarian insulin and IGF-1 receptors, which might lead to follicle stimulation and increased ovarian androgen production.[23] These factors contribute to increased polycystic ovary syndrome in type 1 diabetes, as discussed later in this Review. Additionally, chronic hyperglycaemia might adversely alter folliculogenesis and impair ovarian function in type 1 diabetes, potentially through advanced glycation end-products and their receptors.[4,58]

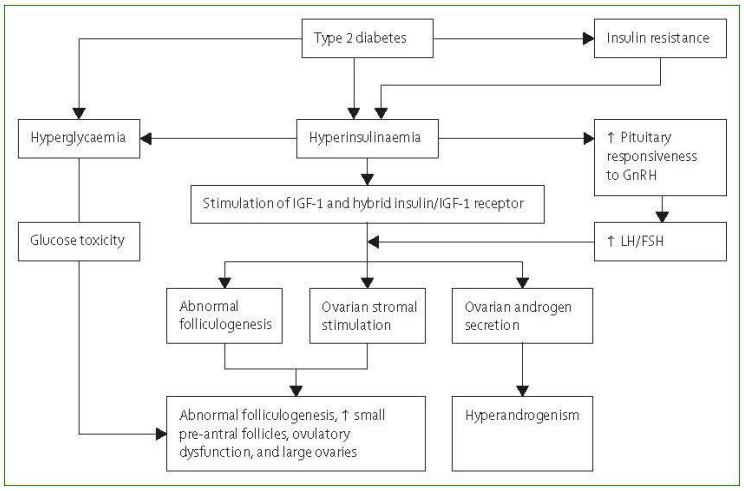

Figure 3: **Mechanisms of interactions between type 2 diabetes and reproductive function**
GnRH=gonadotropin-releasing hormone. IGF-1=insulin-like growth factor 1. LH=luteinising hormone. FSH=follicle-stimulating hormone.

Type 2 diabetes and the ovary

Endogenous insulin resistance and hyperinsulinaemia in type 2 diabetes lead to stimulation of ovarian granulosa cells, increasing recruitment and growth of small follicles. Here, enlarged ovaries with numerous small growing follicles are present and account for the increased prevalence of polycystic ovarian morphology in type 2 diabetes.[59,60] Insulin resistance can also affect ovulation by preventing the recruitment of a large dominant follicle, creating anovulatory states and menstrual disturbances.[61,62] In type 2 diabetes, endogenous hyperinsulinaemia drives hyperandrogenism (figure 3). Indeed, ovarian insulin action on steroidogenesis appears to be preserved, despite systemic insulin resistance in which hyperinsulinaemia has been shown to decrease the expression of ovarian insulin receptors.[63,64] This phenomenon might occur through stimulation of alternative pathways, by insulin binding to the IGF-1 receptor. With advancing duration of type 2 diabetes, β-cell function decreases, and exogenous insulin administration is often required, again exposing the ovary to a high concentration of insulin as outlined for type 1 diabetes. No study has evaluated ovarian function in other monogenic forms of diabetes with decreased insulin secretion, such as maturity onset diabetes of the young. Overall, when ovarian insulin exposure is increased, due to either endogenous or exogenous insulin, follicle stimulation, polycystic ovarian morphology, ovulatory disturbance, and hyperandrogenism can occur.

Polycystic ovary syndrome in diabetes

Polycystic ovary syndrome is the most common endocrinopathy in women of reproductive age, affecting up to 8–13% of women.[65–67] Polycystic ovary syndrome is a heterogenous condition underpinned by insulin resistance and obesity, with diverse reproductive, cardiometabolic, and psychological implications.[66] Diagnosis in adults is on the basis of two of the following criteria: (1) ovulatory or menstrual dysfunction, (2) hyperandrogenaemia, and (3) polycystic ovarian morphology on ultrasound. Given the exogenous insulin treatment of type 1 diabetes, and the endogenous hyperinsulinaemia and insulin treatment of type 2 diabetes, increased prevalence of polycystic ovary syndrome in diabetes is expected, and yet is underappreciated clinically. A detailed review of the clinical implications and management of polycystic ovary syndrome in diabetes is beyond the scope of this Review, and we invite readers to consult the updated, international, evidence-based guidelines by Teede and colleagues.[66]

Polycystic ovary syndrome in type 1 diabetes

Clinicians should be aware of the rising prevalence of polycystic ovary syndrome in adolescent and adult women with type 1 diabetes.[23,68] The pooled prevalence of polycystic ovary syndrome was 24% in women with type 1 diabetes in a meta-analysis,[8] which is considerably higher than that of the general population. We have previously highlighted the hyperandrogenism and ovulatory and menstrual dysfunction that occur secondary to exogenous insulin administration in type 1 diabetes. Intensive insulin therapy is now standard care for type 1 diabetes, improving glycaemia and reducing microvascular and cardiovascular complications, albeit at the price of weight gain. Individuals in the landmark Diabetes Control and Complications Trial (DCCT)[69] who received intensive insulin therapy gained an average of 4·75 kg in 6 years, and were more likely to be overweight than those on conventional therapy. Overall, the increased use of intensive insulin therapy has driven the emergence of polycystic ovary syndrome and the rising prevalence of obesity in people with type 1 diabetes,[70] leading to a vicious circle of insulin resistance, higher insulin doses, and weight gain. It is worth noting that the biochemical and clinical picture of polycystic ovary syndrome in women with type 1 diabetes might be distinct from that in women with polycystic ovary syndrome alone. Women with type 1 diabetes can have increased or normal SHBG, due to low hepatic insulin concentrations, with lower free androgen concentrations than women who have polycystic ovary syndrome without diabetes, in whom SHBG is reduced.[71] Although not previously framed as such, type 1 diabetes might induce what could be termed secondary polycystic ovary syndrome. Polycystic ovary syndrome might also be overlooked in women with type 1 diabetes,[9] and yet, given its impact on reproductive dysfunction, the underlying weight gain, and the adverse metabolic features, polycystic ovary syndrome does need to be considered when managing type 1 diabetes and preventing complications in reproductive-aged women.

Polycystic ovary syndrome in type 2 diabetes

Polycystic ovary syndrome is a substantial contributor to the increasing burden of dysglycaemia and type 2 diabetes

in women.[72–74] Polycystic ovary syndrome was associated with increasing the probability of developing type 2 diabetes by a factor of three in a meta-analysis, with other pertinent risk factors being Asian ethnicity and obesity.[75] Polycystic ovary syndrome is not only highly prevalent in women with type 2 diabetes,[76] but appears to herald an earlier onset of type 2 diabetes in women with polycystic ovary syndrome than in women without.[75,77] Polycystic ovarian morphology (a feature of polycystic ovary syndrome) is reportedly more common in type 2 diabetes, with prevalence as high as 61% in Indian women[27] and 34% in Turkish women.[78] As previously discussed, obesity, insulin resistance, hyperinsulinaemia, type 2 diabetes, and polycystic ovary syndrome all have a combined and inter-related effect on reproductive function, but the evidence is not sufficient to tease out the relative contribution of each condition to reproductive dysfunction. Further large, longitudinal cohort studies are needed to understand these relationships.

Regardless of whether the causation of polycystic ovary syndrome is primary or secondary to endogenous (type 2 diabetes) or exogenous hyperinsulinaemia (for both types of diabetes), the interaction between polycystic ovary syndrome and diabetes and the effect on reproductive health require greater recognition and management. Likewise, promoting a healthy lifestyle and weight management should be further emphasised in the care of affected women (panel 1).

Panel 1:* Summary of polycystic ovary syndrome management in diabetes

- Clinicians should routinely review insulin doses, weight gain, BMI, and waist circumference in women with diabetes, with a focus on preventing excess weight gain
- In women with type 1 and type 2 diabetes who have menstrual dysfunction, hyperandrogenism, or obesity, polycystic ovary syndrome should be considered
- Screening of women with polycystic ovary syndrome for type 2 diabetes is recommended
- Annual assessment of cardiovascular risk factors in women with diabetes and polycystic ovary syndrome is encouraged
- Lifestyle interventions, potentially combined with the oral contractive pill, are recommended for management of menstrual dysfunction and hyperandrogenism
- The use of metformin can be considered in women with type 1 or type 2 diabetes and polycystic ovary syndrome, with proven metabolic benefits in polycystic ovary syndrome

*Based on the international evidence-based guidelines by Teede and colleagues.[66]

Fertility

Fecundity is defined as the natural potential to reproduce and give birth to live offspring, whereas fertility relates to reproductive fitness in terms of offspring born per couple. In general, the female reproductive period starts with the onset of menarche and ends at menopause, although both fecundity and fertility generally conclude before the onset of menopause, around the age of 40–45 years.[79]

Type 1 diabetes and fertility

Few studies have evaluated fecundity in women with type 1 diabetes, although some studies report a reduced number of livebirths in women with type 1 diabetes, with a recent Taiwanese study showing a lower rate of livebirths for women with type 1 diabetes than for matched, non-diabetic controls.[80] An international study by the Type 1 Diabetes Genetics Consortium[81] showed that people with type 1 diabetes had statistically significantly fewer offspring than their unaffected siblings. This effect was more prominent in women and individuals with childhood-onset disease. Similar findings were reported in a Finnish cohort study, in which men and women with childhood-onset type 1 diabetes had fewer livebirths than matched controls.[6]

A Swedish population-based study of women hospitalised for type 1 diabetes between 1965 and 2004 showed that the standardised fertility ratio (SFR; the ratio of observed to expected number of livebirths) was reduced in women with type 1 diabetes, and even more so in the presence of microvascular and cardiovascular complications. However, whether women with complications related to type 1 diabetes choose to have fewer pregnancies or, conversely, whether poor metabolic control directly affects fertility remains unclear. Nonetheless, reduced SFR was confined to women first hospitalised before 1985, suggesting that improved contemporary diabetes control might ameliorate infertility,[82] potentially via the hypothalamic–pituitary–gonadal axis. Reduced fertility in type 1 diabetes is also likely to be related to increased exogenous insulin use, so-called secondary polycystic ovary syndrome, and rising BMI, as we outlined earlier. Even after adjustment for irregular menses, the risk of infertility remains increased in type 1 diabetes.[83] Although assisted reproductive technology use is very common in polycystic ovary syndrome,[66,84] it is unclear whether its use is also increased in women with type 1 diabetes.

Type 2 diabetes and fertility

Fewer studies have explored fertility rates in women with type 2 diabetes than in women with type 1 diabetes. Isik and colleagues[85] reported higher concentrations of FSH and lower ovarian volumes in women with type 2 diabetes than in age-matched controls, indicating a lower degree of ovarian reserve. An observational, descriptive study of 256 married women with type 2 diabetes attending a university hospital clinic in Iran showed a high rate of fertility, with an average of five pregnancies per participant. However, this group had an unexpectedly low prevalence of polycystic ovary syndrome and

hyperandrogenaemia.[86] Given the overlap with polycystic ovary syndrome, infertility rates and assisted reproductive technology use could be expected to be higher than was observed, but research in this area is scarce.

Fecundity in type 1 and 2 diabetes

Overall, there is a paucity of studies directly examining fecundity in women with both types of diabetes. A cohort study of pregnant women enrolled between 1999 and 2008 showed that fecundability rates in women with both types of diabetes were decreased, compared with rates in women without diabetes.[87] Fecundability odds ratios were reduced by 24% and 36% for women with type 1 and type 2 diabetes, respectively, in comparison with women without diabetes, and time-to-pregnancy was increased in women with both type 1 and type 2 diabetes, despite adjustments for BMI, cycle length, and regularity.[87] Again, this decrease in fecundability might overlap with the high prevalence of polycystic ovary syndrome, which is known to impair fecundity and fertility. More research is also needed in this area.

Preconception care

Pregestational diabetes is associated with an increased risk of adverse maternal and fetal outcomes.[88] Preconception care is vital to prevent adverse pregnancy outcomes, optimise lifestyle factors, and reduce diabetes-related distress.[89] This care entails contraception, family planning, optimisation of metabolic control, complication screening, lifestyle and weight management, and usual diabetes care.

Preconception care in type 1 diabetes

Pregnancy planning for women with type 1 diabetes benefits glycaemic control and reduces perinatal mortality, congenital malformations, and preterm labour.[90,91] The International Society for Pediatric and Adolescent Diabetes[92] and the American Diabetes Association[93] recommend that preconception care should start at the beginning of puberty and be maintained throughout the reproductive lifespan. A specific programme for preconception care in adolescents with type 1 diabetes has shown long-lasting benefits to reproductive health behaviours and outcomes,[94] although the uptake and implementation of these guidelines in clinical practice is unknown. A 15-year study assessing the effect of preconception care on type 1 diabetes pregnancy outcomes from 1998 to 2012 showed slight improvements in glycaemic control with increased use of insulin analogues and insulin pumps.[95] Despite this improvement, the proportion of women planning pregnancy did not change during this time. Kohn and colleagues[96] showed that a substantial proportion of health-care providers were reluctant to address contraception and preconception care in adolescent girls with type 1 diabetes, citing discomfort, insufficient time, and inadequate subject knowledge as barriers. A study evaluating ovulation for 6 months in adolescents with type 1 diabetes and in healthy controls showed that those with insufficient metabolic control were still ovulating despite elongated cycles,[97] highlighting potential fertility and the need for preconception counselling regarding the risks of unplanned pregnancy in women with type 1 diabetes, irrespective of the presence of menstrual disturbances or poor glycaemic control.

Preconception care in type 2 diabetes

The burden of diabetes in pregnancy is rising because of the growing incidence of type 2 diabetes in reproductive-aged women, in parallel with current obesity rates and a trend towards an increasing age of childbearing.[98] The National Pregnancy in Diabetes audit reported a doubling of the proportion of type 2 diabetes pregnancies over the past decade, from 27% to 50%.[99] Pregnancy planning improves peripregnancy glycaemic control and improves perinatal outcomes in this cohort.[88] Even though preconception care is recognised as part of standard care for type 1 diabetes, young women with type 2 diabetes do not have access to similar programmes, and are consequently at high risk of complications in pregnancy. The treatment options for type 2 diabetes in adolescents and youth (TODAY) study[100] showed a 10% rate of unplanned pregnancies in adolescents with type 2 diabetes, despite preconception education, which highlights the need to better engage and inform young women with type 2 diabetes regarding their risk of adverse pregnancy outcomes. Women with type 2 diabetes are less likely to access preconception care, and often have lower awareness of pregnancy risks in type 2 diabetes and less effective contraception than women without diabetes.[101] Socioeconomic disparity, cultural factors, and religious factors can also present barriers to women accessing contraception and prepregnancy care.[102]

Preconception care in type 1 and type 2 diabetes

During the past two decades, increased attendance rates at preconception care clinics have resulted in increased folate supplementation[101] and reduced prevalence of stillbirths and congenital anomalies in women both with type 1 and type 2 diabetes.[88,103,104] However, the uptake of preconception care services remains suboptimal,[101] with one in four women with type 1 diabetes, and one in two with type 2 diabetes not establishing first contact with joint diabetes and antenatal clinics in early pregnancy.[99]

A cohort study of 440 pregnancies in 220 women with pregestational type 1 or type 2 diabetes showed elevated recurrent adverse pregnancy outcomes, with no improvements observed in preparation for subsequent pregnancies, despite previous pregnancy complications. Notably, the median interpregnancy interval in this cohort was only 12 months, representing a short window of opportunity for preconception optimisation,[105] and emphasising the need for ongoing post-partum

management and engagement with women who are at high risk. Greater insights into the reach, adequacy, barriers, and implementation strategies in preconception care in both types of diabetes are now needed.

Pregnancy in women with type 1 and type 2 diabetes

Women with both types of diabetes have an increased risk of pregnancy complications, including pregnancy loss, pre-eclampsia, and large-for-gestational-age neonates.[106–109] Although a comprehensive review of all maternal and fetal outcomes in type 1 and type 2 diabetes pregnancies is beyond the scope of this Review (and can be found in a review by Schaefer-Graf and colleagues[110]), we discuss key areas associated with type 1 and type 2 diabetes, including pregnancy loss, large-for-gestational-age neonates, and macrosomia.

Pregnancy loss

Pregestational diabetes has historically been associated with increased perinatal mortality, including spontaneous abortion and stillbirth. Type 1 diabetes is associated with a five times greater risk of stillbirth and a ten times greater risk of congenital malformation than that of the general population.[111] Suboptimal glycaemic control has been consistently associated with an increased risk of pregnancy loss in type 1 diabetes,[112,113] for which the predominant underlying causes appear to be major congenital anomalies and prematurity.[114] Although the prevalence of spontaneous abortion and stillbirth in women with type 1 diabetes appears to be declining with improvements in diabetes care,[115] the risk of pregnancy loss in type 1 diabetes remains considerably higher than that of the general population.[116]

Infants of women with type 2 diabetes in the UK had two times the risk of stillbirth, 2·5 times the risk of a perinatal mortality, and 11 times the risk of congenital malformations compared with regional and national figures.[117] Similar to women with type 1 diabetes, glycaemic control is an important determinant of perinatal mortality in this cohort,[118] although the increased prevalence of advanced maternal age and obesity are likely to magnify the risk of adverse fetal outcomes in women with type 2 diabetes.[114] Furthermore, women with polycystic ovary syndrome appear to have a higher risk of pregnancy loss than women without this condition, which might contribute to increased risk in both types of diabetes.[66]

Contemporary studies have not reported substantial differences in perinatal mortality rates between type 1 and type 2 diabetes, although pregnancy loss is more prevalent in the first trimester in type 1 diabetes and in the third trimester in type 2 diabetes.[119] Studies evaluating placental histology in women with pregestational diabetes have reported a high prevalence of placental infarcts in women with type 2 diabetes, which is consistent with a vascular cause, rather than the glycaemic cause observed in women with type 1 diabetes, who had a higher risk of abnormal placental development.[120,121]

Macrosomia and large-for-gestational-age infants

Macrosomia and large-for-gestational-age infants are prevalent in women with diabetes, with up to 50% of infants born to mothers with type 1 diabetes having a birthweight above the 90th percentile.[122] Suboptimal glycaemic control before and during pregnancy, particularly in the third trimester, is a significant predictor of fetal macrosomia.[123] However, the prevalence of macrosomia (defined as birthweight >4000 g) remained elevated at 23% in a Polish type 1 diabetes cohort,[124] despite apparent good glycaemic control throughout pregnancy, which suggests that other factors outside of glycaemic control could play a role in fetal growth. Studies have shown trends towards increasing maternal BMI and excessive gestational weight gain in women with type 1 diabetes, both of which have been consistently associated with large-for-gestational-age newborns and macrosomia in type 1 diabetes.[123,125]

Up to 30–40% of women with type 2 diabetes have a newborn baby who is large-for-gestational age.[122] They have a comparatively shorter duration of disease, and therefore often have lower HbA_{1c} than women with type 1 diabetes. Nevertheless, this advantage appears to be negated by the rising trends of maternal overweight and obesity. Parellada and colleagues[126] found that nearly half of women with type 2 diabetes had excessive gestational weight gain, which was independently associated with higher infant birthweight, regardless of prepregnancy BMI, HbA_{1c}, ethnicity, and parity. With excessive gestational weight gain (exceeding the Institute of Medicine guidelines), the risk of large-for-gestational-age neonates or macrosomic infants and caesarean delivery is doubled in women with type 2 diabetes.[127] Independent of maternal BMI, studies suggest excessive gestational weight gain is increasingly common in women in the general population, and in women with both types of diabetes.[126,128,129] Emphasis on healthy lifestyle and weight management before pregnancy and the prevention of excessive gestational weight gain is pivotal for optimising fetal outcomes in pregnancies for both types of diabetes.

Pre-eclampsia

Pre-eclampsia affects 2–7% of pregnancies and is a leading cause of maternal and fetal morbidity and mortality. The risk of pre-eclampsia is amplified by two to five times in women with diabetes, particularly in women with type 1 diabetes.[130,131] Poor glycaemic control appears to be a reliable predictor of pre-eclampsia. A meta-analysis of type 1 diabetes pregnancies showed that odds of pre-eclampsia were increased by 1·5 times for each percentage increase in HbA_{1c} level.[132] In general, birth outcomes are less favourable in pregnancies of women with type 1 diabetes pregnancies than in pregnancies of

women with type 2 diabetes, although these outcomes vary considerably between different diabetes antenatal services.[103] Women with type 1 diabetes are at greater risk of hypoglycaemia than women with type 2 diabetes, and could face more difficulty maintaining normoglycaemia[102] with the changing insulin sensitivities of pregnancy. Furthermore, maternal obesity and pre-existing vasculopathy increase the risk of abnormal placentation and pre-eclampsia.[131,133,134] Again, these elevated risks highlight the importance of optimising weight and metabolic risk factors before conception. Consequently, the adverse outcomes associated with pre-eclampsia could persist beyond pregnancy, when an increased risk of developing retinopathy or nephropathy has been observed in women with type 1 diabetes with a history of pre-eclampsia.[135,136]

Women with type 2 diabetes have twice the risk of pre-eclampsia compared with the general population.[131] Obesity and polycystic ovary syndrome are common in women with type 2 diabetes, and both conditions are shared risk factors for pre-eclampsia.[137,138] However, the extent to which obesity and polycystic ovary syndrome independently contribute to adverse maternal and fetal outcomes is unclear. Findings from a large, Swedish cohort study suggest that maternal obesity exacerbates pre-eclampsia risk in type 1 diabetes, but not in type 2 diabetes.[131] Even so, considering the associations between maternal BMI, polycystic ovary syndrome, type 2 diabetes, and the overall risk of pregnancy complications, women should ideally aim for a healthy weight before pregnancy, regardless of their diabetes status.

Principles of diabetes management in pregnancy

Management of both types of diabetes in pregnancy should incorporate lifestyle interventions to optimise preconception weight, prevent excessive gestational weight gain, and improve glycaemic control. This management should occur in the setting of multidisciplinary care with regular self-monitoring of blood glucose and individualised therapy to optimise metabolic control. Although the principles of pregnancy management are similar in both types of diabetes, the unique causality and risk profiles need clear consideration, along with other risk factors, to provide individualised management and counselling.[122]

The association between poor glycaemic control and adverse maternal, fetal, and neonatal complications is well established.[118] Observational studies of women with pre-existing diabetes in pregnancy have shown that the lowest risk of adverse fetal outcomes is associated with HbA_{1c} of less than 6·5% (42–48 mmol/mol) early in gestation and in subsequent trimesters.[119] Of note, the glycaemic levels at which pregnancy complications occur might be different in type 1 and type 2 diabetes, with adverse perinatal outcomes occurring at lower HbA_{1c} values in women with type 2 diabetes.[133,139] A meta-analysis of pregnancy outcomes showed that despite less severe dysglycaemia, maternal and neonatal morbidity are no different in women with type 2 and type 1 diabetes.[139] These findings suggest that factors independent to glucose, such as increased maternal age, obesity, unfavourable metabolic profile, and low socioeconomic status in women with type 2 diabetes,[122,139] might offset better glycaemic control in pregnancy.

HbA_{1c} and self-monitoring of blood glucose targets for optimal glycaemic control from the American Diabetes Association guidelines are summarised in panel 2. As HbA_{1c} and self-monitoring of blood glucose might not adequately capture glycaemic variability, continuous blood glucose monitoring devices have been used in pregnancy. The CONCEPTT trial[140] showed a reduction in glycaemic variability and adverse fetal outcomes in women with type 1 diabetes, although there was no benefit to maternal hypoglycaemia.[140] Further research is needed on the utility and efficacy of continuous blood glucose monitoring before its advocacy in pregnancy. Although the use of insulin analogues (eg, insulin lispro, insulin aspart, insulin glargine, and insulin detemir) has been shown to ameliorate glucose excursions and hypoglycaemia, evidence regarding the safety and efficacy of newer insulin analogues and concentrated insulin preparations is scarce.[110] Presently, there is no evidence to support the use or benefits of more expensive, continuous subcutaneous insulin infusion in pregnancy over those of multiple daily injections of insulin,[141] although further research is underway. Maternal obesity[131,142] and excessive gestational weight gain[129] are modifiable risk factors that can contribute to adverse pregnancy outcomes, independent of diabetes. Therefore, lifestyle interventions are fundamental to manage preconception weight and control gestational weight gain to improve pregnancy and long-term metabolic health outcomes in women with both types of diabetes. Gestational weight gain should be closely monitored, particularly during the rapid escalation of insulin doses during pregnancy. Clinical recommendations for pregnancy care in type 1 and type 2 diabetes are outlined in panel 2.

Menopause

Menopause is characterised by the permanent cessation of menstruation and ovulation due to the depletion of ovarian reserves. Natural menopause occurs on average at around age 51 years, ranging from 40 to 62 years in high-income countries. The large variation in menopausal age reflects the variability in ovarian ageing.[143] Although genetic variants are known to contribute to about 50% of the variation in age at menarche and menopause,[144] multiple factors—including hormonal and environmental exposures, socioeconomic status, and stress throughout the life course, can affect the timing of menarche and menopause. The biological processes that govern the timing of the beginning and end of reproductive life are not well understood.

Type 1 diabetes and menopause

There has long been speculation about early menopause in women with type 1 diabetes. Several hypotheses have been put forward, including accelerated ovarian ageing (mediated by sustained dysglycaemia and premature vascular ageing[145]), autoimmune oophoritis, and the depletion of ovarian follicles secondary to insulin stimulation.[146,147] Several studies suggest that the depletion of ovarian follicles in type 1 diabetes occurs at a faster rate than in healthy controls. Whereas in the early reproductive years, polycystic ovarian morphology, polycystic ovary syndrome, and AMH are increased, lower serum AMH concentrations have been reported in women with type 1 diabetes in the fourth decade of life, which is consistent with the postulated accelerated decline in ovarian reserves.[147,148]

A study of adults with type 1 diabetes and their family members, by the Familial Autoimmune and Diabetes Study, found that women with type 1 diabetes were more likely to have menarche later and enter menopause earlier than their sisters without diabetes and unrelated healthy controls, resulting in a 6-year (approximately 17%) reduction in reproductive years.[7] Both type 1 diabetes status and menstrual irregularities before age 30 years were independent determinants of earlier menopause. This study evaluated women diagnosed with type 1 diabetes between 1950 and 1965, which suggests that they were not likely to have received intensive insulin therapy. Studies of type 1 diabetes in the modern era of intensive insulin therapy have found a milder effect of type 1 diabetes status on age of natural menopause. A large, European longitudinal study investigated the association between diabetes and age of natural menopause in more than 250 000 women enrolled between 1992 and 2000, finding no statistically significant association with diabetes.[149] However, when the age of diabetes onset was taken into account, women diagnosed with diabetes before the age of 20 years reached menopause earlier than women without diabetes. These associations were unchanged after adjusting for age, smoking, and other reproductive factors. Although this study did not discriminate between women with type 1 versus type 2 diabetes, a reasonable assumption can be made that most women diagnosed with diabetes before age 20 years had type 1 diabetes.

A cross-sectional analysis of Dutch women with type 1 diabetes, recruited between 1993 and 1997, showed that the mean age of natural menopause in affected women was no different from that in women without diabetes (49·8 years in both groups).[145] No association of type 1 diabetes with earlier menopause was detected after adjustment for age, smoking, and parity. A plausible explanation for the reduced age of natural menopause in contemporary cohorts of women with type 1 diabetes could relate to the improved glycaemic control with modern therapies. Surprisingly, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, a follow-up of the landmark Diabetes Control and

Panel 2:* Recommendations for preconception and pregnancy care in type 1 and type 2 diabetes

Preconception

- Preconception counselling should be incorporated into routine diabetes care, starting at puberty and continuing for all women with reproductive potential, ideally delivered by a multidisciplinary team
- Effective contraception should be prescribed and used until ready for pregnancy
- Healthy lifestyle and healthy weight should be promoted
- Preconception health should be optimised (eg, folate use, and alcohol and smoking cessation)
- Intensification of glycaemic control as close to normal as is safely possible, ideally less than 6·5% (48 mmol/mol), to reduce adverse fetal and maternal outcomes
- Complication screening and management should be up to date
- Women who are pregnant or planning pregnancy should be counselled on the risk of development and progression of diabetic retinopathy, and dilated eye examinations should occur ideally before conception or in the first trimester, with monitoring at every trimester thereafter

Glycaemic targets in pregnancy

- Fasting and postprandial (and possibly also preprandial testing for women on insulin pumps or basal-bolus therapy) self-monitoring of blood glucose recommended
- Fasting for less than 5·3 mmol/L (95 mg/dL)
- 1-h postprandial for less than 7·8 mmol/L (140 mmol/dL)
- 2-h postprandial for less than 6·7 mmol/L (120 mmol/dL)
- Ideal HbA_{1c} target in pregnancy is less than 6% (42 mmol/mol) or 7% (53 mmol/mol) if hypoglycaemia is a concern
- Individualised treatment targets should account for maternal hypoglycaemic risk

Management of type 1 and type 2 diabetes in pregnancy

- Insulin is the preferred agent for management of both types of diabetes in pregnancy, although no specific insulin regimen is recommended
- The use of low-dose aspirin (60–150 mg/day) is recommended from the end of the first trimester until delivery to lower risk of preeclampsia in women with both types of diabetes who are at high risk
- Review and cease potentially teratogenic medications
- Recommended gestational weight gain during pregnancy is 11·5–16 kg (25–35 lb), 6·5–11 kg (15–25 lb), and 5–9 kg (10–20 lb) in healthy weight, overweight, and obese women, respectively

Type 1 diabetes

- Intensification of treatment to achieve metabolic goals
- Increased risk of hypoglycaemia and impaired awareness of hypoglycaemia in the first trimester
- Education on risk of diabetic ketoacidosis at lower blood glucose thresholds than in non-pregnant state
- Rapid tightening of glycaemic control can worsen pre-existing retinopathy

Type 2 diabetes

- Lifestyle and weight management, in conjunction with optimisation of metabolic control, should be the first-line therapy
- Insulin is the preferred agent for treating hyperglycaemia
- Oral agents (metformin and glibenclamide) cross the placenta and are generally insufficient to overcome insulin resistance in type 2 diabetes, and there is no long-term safety data for them

*Adapted from the American Diabetes Association (ADA) clinical practice guidelines for standards of medical care in diabetes for 2019.[88]

Complications Trial (DCCT), reported no difference in age of natural menopause (49·9 *vs* 49·0 years, p=0·28) for the intensive insulin therapy and conventional treatment groups. In addition, HbA_{1c} or the presence of microvascular complications did not affect age of natural menopause.[150] In contrast to these findings, Sjoberg and colleagues[151] observed that women with type 1 diabetes who had end-stage renal disease and proliferative retinopathy were at increased risk of earlier menopause compared with the general population. These discrepancies can probably be explained by the DCCT and EDIC cohorts achieving better glycaemic control than other observational type 1 diabetes cohorts, in addition to the relatively low prevalence of substantial microvascular disease. Poorer diabetes control might therefore affect the age of natural menopause, and the presence of severe microvascular complications in women with long-term diabetes—underpinned by long-standing metabolic disruption—could herald the onset of early menopause in this cohort.

Type 2 diabetes and menopause

Oestrogen is an important hormone for regulation of body fat distribution and insulin sensitivity. Metabolic changes during the menopausal transition include a decrease in physical activity and energy expenditure, leading to weight gain, in addition to redistribution of body fat, particularly around the abdominal region.[152] Although weight gain can be age-related, the increased abdominal adiposity observed in menopause appears to be independent of ageing.[153] Impairment of insulin secretion and insulin sensitivity can also occur during this period, leading to a potentially increased risk of developing type 2 diabetes.[154,155] A Chinese study revealed that for every 1-year delay in menopausal age, the presence of diabetes was reduced by 2%.[156] Compared with women whose menopausal age was 46–52 years, those who had experienced early menopause (before age 45 years) had a 20% higher incidence of type 2 diabetes.[156] An analysis of the Women's Health Initiative study,[157] which examined a large cohort of postmenopausal women, concluded that women with a reproductive lifespan (the difference between age at menarche and age at final period) of less than 30 years had a 37% higher risk of type 2 diabetes than women with a reproductive lifespan of 36–40 years. These results remained significant after adjustment for chronological age, age at menarche, and cycle regularity.[157] Whereas early menopause appears to be associated with heightened risk of type 2 diabetes, several observational and longitudinal studies have consistently reported that type 2 diabetes risk is not associated with natural menopause occurring at the average age, even after adjustment for confounders such as age, metabolic risk factors, BMI, and smoking.[158]

Few studies have evaluated age of natural menopause in women with type 2 diabetes because of the late onset of disease, which typically occurs after menopause. A large study of women with type 2 diabetes from Latin America found that women with type 2 diabetes who were younger than 45 years had three times the risk of early menopause compared with their counterparts without diabetes.[159] In this study, women with type 2 diabetes had an elevated propensity towards deterioration in quality of life because of climacteric symptoms, although this risk was attenuated after adjusting for confounders. Studies of women with type 2 diabetes—a more heterogeneous group than type 1 diabetes—do not consistently show biologically relevant differences in age at menopause compared with healthy women, although there might be a trend towards earlier menopause.[146]

Menopausal hormone therapy in women with type 1 and type 2 diabetes

The menopausal transition is associated with adverse metabolic changes and reduced insulin sensitivity, which can exacerbate dysglycaemia in women with established type 1 or type 2 diabetes.[155] Although data on the effect of menopause in women with established diabetes are scarce, evidence from several clinical trials consistently show a reduction in type 2 diabetes risk with the use of menopausal hormone therapy (MHT). Furthermore, MHT might improve the metabolic profile and glucose homoeostasis in women with established diabetes,[154,158] and improve climacteric symptoms and bone mineral density.

The literature surrounding MHT use in women with type 1 diabetes is scarce, and the following discussion of MHT use in diabetes therefore relates primarily to those with pre-existing type 2 diabetes. Women with type 2 diabetes are half as likely to be users of MHT compared with their counterparts without diabetes, which could be attributable to concerns regarding the cardiovascular safety of MHT in this cohort.[154] Several small randomised controlled trials of short duration have reported benefits to glycaemia and lipid profile with the use of conjugated oestrogens alone[160] or in combination with medroxyprogesterone acetate[161] in women with type 2 diabetes. Continuous combined oral oestradiol and noresthisterone[162,163] improved serum cholesterol and BMI, but had no effect on glycaemia or triglycerides. Transdermal oestrogen alone or with cyclical dydrogesterone[164] was associated with significant improvements in HbA_{1c} and lipid profile in a small study of 28 women with type 2 diabetes, over 12 months of follow-up. A 6-month randomised controlled trial of transdermal oestrogen in combination with continuous noresthisterone[165] did not alter glycaemic control in type 2 diabetes, but benefited serum cholesterol and triglycerides, and decreased factor VIII activity, which might translate to reduced coronary thrombotic risk. However, head-to-head comparisons of oral versus transdermal MHT have yielded either beneficial or neutral effects on glycaemia.[158]

The decision to commence MHT, and the type of regimen and dosing, should take into account the

	Summary of findings		Clinical implications	Gaps and key future research focus areas
	Type 1 diabetes	Type 2 diabetes		
Menarche and menstrual disturbance	Delayed puberty evident in pre-pubescent type 1 diabetes before intensive insulin therapy;[10] menarche normalises with minor delays, despite contemporary treatment;[14–17] menstrual disturbance common; associated with poor metabolic control;[19,25] links to increased risk of microvascular sequelae;[18] overlap with obesity and PCOS[8,26]	Scarce data; 21% prevalence of menstrual irregularity in adolescents in one study;[29] menstrual disorders overlap with rising obesity and PCOS[28,29,31]	Might be secondary to functional hypogonadism in type 1 diabetes, which also has adverse effects on bone health; menstrual disorders are related to poor metabolic control and complications;[25] PCOS is common and needs screening and management; rising BMI and obesity make healthy lifestyle management a priority	Clarify menstrual disorders, hypogonadism, cardiovascular and skeletal links, and outcomes in type 1 diabetes; prevalence of menstrual disorders in young women with type 2 diabetes and relationship with obesity and PCOS; effect of menstrual disturbance on quality of life in women with diabetes
PCOS	Increased prevalence of PCOS (pooled prevalence of 24% in a meta-analysis);[8] rising BMI and obesity with intensive insulin therapy in type 1 diabetes exacerbates PCOS[9] Intensive insulin therapy drives follicle development, polycystic ovarian morphology, hyperandrogenism, and PCOS[23,71]	Type 2 diabetes prevalent in PCOS;[74] earlier onset of type 2 diabetes in women with PCOS;[76,77] PCOS in type 2 diabetes suggestive of a more severe phenotype of PCOS[166]	Awareness needed on PCOS in type 1 and type 2 diabetes; interaction between PCOS and type 2 diabetes exacerbated by rising BMI and obesity; need to understand that intensive insulin therapy has an ovarian impact, both directly and via rising BMI; multidisciplinary care, lifestyle, and weight management, potentially in combination with the oral contraceptive pill or metformin for treatment of menstrual disorders and hyperandrogenism[66]	Clarify if PCOS is primary or secondary in diabetes; understand fertility and its relationship to PCOS in diabetes; optimal treatment of type 1 and type 2 diabetes in PCOS; genetic and metabolic characterisation of type 1 diabetes and PCOS; long-term cardiovascular outcomes in diabetes and PCOS
Fertility	Lower livebirth rates;[6,80–82] increased risk of infertility,[83] cause still unclear	Scarce data; lower ovarian reserves in a single small study[85]	Infertility could be associated with young onset of type 1 diabetes;[81,82] concomitant autoimmune conditions might contribute to subfertility in type 1 diabetes;[80] PCOS might underlie subfertility in both types of diabetes	Fecundity in both types of diabetes is unclear; mechanisms underlying reproductive issues in type 1 diabetes; fertility outcomes and treatment responses in type 1 and type 2 diabetes; use of AMH in predicting reproductive outcomes in type 1 and type 2 diabetes
Preconception care	Structured preconception care reduces perinatal mortality and morbidity in type 1 diabetes;[109] increased use of insulin analogues and personal insulin pumps over the years is accompanied by a slight improvement in glycaemic control[95]	Preconception care improves glycaemic control and reduces perinatal mortality and risk of large-for-gestational-age infants;[88] poor uptake of preconception care in type 2 diabetes, related to socioeconomic disparity and care access[100]	Preconception care in diabetes is suboptimal, with poor guideline implementation;[96,100,101] routine preconception care needed in both types of diabetes; screen for and manage complications	Develop strategies for engagement, interactive care, and healthy lifestyle; explore and address barriers to provision of preconception care
Pregnancy outcomes	Pregnancy loss more prevalent in first trimester, related to glycaemic control;[113,119,120] increased macrosomia and pre-eclampsia;[121] pre-eclampsia in pregnancy portends increased risk of microvascular complications;[134,135] increasing maternal BMI associated with large-for-gestational-age neonates and macrosomia[122,124]	Pregnancy loss more prevalent in third trimester, related to vascular issues;[113,119,120] adverse pregnancy outcomes occur at lower HbA_{1c} than in type 1 diabetes, in part related to high BMI[125,138]	Adverse pregnancy outcomes still high in both types of diabetes, despite contemporary care;[102,103,106,107,109,122,132] increased maternal BMI, excessive large-for-gestational-age neonates, and higher maternal age (in type 2 diabetes) are likely to negate improvements that are due to better glycaemic control[130,138,141]	Role of modern technology (eg, continuous blood glucose monitoring and closed-loop systems) in type 1 diabetes pregnancies; determine effects of obesity and diabetes on early placental development; understand and prevent transgenerational effect of obesity and diabetes
Menopause	Earlier menopause;[7,148] scarce data on cardiovascular and metabolic outcomes of MHT in type 1 diabetes[165]	Risk of early menopause in type 2 diabetes unclear; majority of short-term studies show a neutral or positive effect of MHT on metabolic outcomes[157,159–164]	Menopausal age might be reduced in type 1 diabetes; health effects of menopause in both types of diabetes needs to be explored; effects of MHT on metabolic outcomes; MHT can be used with low cardiovascular risk	Effect of earlier menopause in type 1 diabetes on fracture risk and bone health; hormone therapy in both types of diabetes; long-term cardiovascular effects from glycaemic control in both types of diabetes; efficacy of MHT in both types of diabetes

PCOS=polycystic ovary syndrome. AMH=anti-Müllerian hormone. MHT=menopausal hormone therapy.

Table: Summary of reproductive disturbance in diabetes

individual's cardiometabolic risk profile and preferences. Guidelines for prescribing MHT for postmenopausal women with diabetes, particularly for type 1 diabetes, are scarce. A Cochrane review[166] identified only one small study,[162] which compared cardiovascular risk factors after 12 months of MHT in women with type 1 and type 2 diabetes, detecting no differences between the groups. Even though MHT appears to have no clear adverse effects on metabolic variables in women with type 1 or type 2 diabetes, evidence to support long-term cardiovascular safety of MHT in this cohort is inadequate, and an overall strategy incorporating lifestyle modifications should be considered, including weight management and smoking cessation. Transdermal oestrogen and micronised progesterone (or a less metabolically active progesterone) might be preferable over oral regimens,

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1.2.7 Summary and knowledge gaps

Reproductive dysfunction is highly prevalent in women with T1D, and encompass a broad spectrum of presentations, including menarchal and pubertal delay, subfertility and early menopause. An estimated 40% of women with T1D will encounter reproductive dysfunction in their lifetime[41], yet this is not routinely addressed in the clinical setting, suggesting that a greater focus on women's health is needed in contemporary T1D management.

Historically, women with T1D faced pubertal and menarchal delay, hypothalamic amenorrhea and infertility as a result of insulin deficiency. Enhanced metabolic control has ameliorated, but not completely eradicated these problems. The majority of studies relating to menstrual disorders in T1D have been in adolescents, and the characterisation of menstrual dysfunction in women with T1D under contemporary therapy, is unclear. Furthermore, the rise of PCOS in this cohort in the last two decades suggest that ovarian dysfunction may also arise from intensification of insulin therapy for glycaemic control. It is not clear if insulin doses and glycaemic control directly correlate to the degree of hyperandrogenaemia. Additionally, secular trends demonstrate the rise of obesity in individuals with T1D, which may predispose to menstrual irregularity and PCOS. Both PCOS and T1D are associated with increased CVD risk; however, the long-term metabolic outcomes in women with PCOS and T1D are yet to be uncovered.

Fertility in women with diabetes is an under-studied area. In general, women with T1D appear to have fewer offspring, particularly in those with microvascular complications. However, the aetiologies of infertility and/or subfertility in T1D remains to be delineated. The higher prevalence of PCOS and hyperthyroidism in this cohort may contribute to, or exacerbate fertility problems. Menopause in women with T1D appears to be earlier than their non-diabetic counterparts; however, the impact of earlier menopause on fracture risk and bone health in this cohort is not known. Lastly, evidence regarding the efficacy and safety

profile of menopausal hormone therapy (MHT) in women with T1D is scarce, yet much needed to guide clinical practice.

Given these knowledge gaps, my work aims to evaluate associations between female reproductive, metabolic and bone health in T1D. I have led the writing of an invited comprehensive review of reproductive disorders in women with diabetes, in *The Lancet Diabetes and -Endocrinology*, bringing on board world leaders in female reproductive health. This manuscript provides context for my thesis, and encompasses learnings from my research.

1.3 THE BONE-REPRODUCTIVE AXIS IN T1D

The skeletal and reproductive health systems are intricately linked. Osteoporosis is a substantial public health problem, with the major cause attributable to oestrogen deficiency following menopause in women. Mendelian randomization studies have shown a potential causal role in osteoporosis development, where every additional year of age at menarche is associated with a reduction in femoral neck and lumbar spine BMD[77]. Oestrogen is the dominant sex steroid involved in the regulation of skeletal growth and bone metabolism in women. It is now firmly established that the cell types in bone, osteoblasts, osteocytes and osteoclasts, express functional oestrogen receptors (ERs), which mediates most of oestrogen's actions on bone cells. Randomised controlled trials have shown that oestrogen therapy given to postmenopausal women was able to augment BMD at multiple skeletal sites and reduce hip fracture risk by 33%, compared with placebo[78].

Bone remodelling is a process by which aging bone is replaced by new tissue, through the coordinated actions of osteoblasts and osteoclasts. The activities of these cells are combined into defined anatomical spaces, known as basic multicellular units (BMUs), or bone remodelling units. Bone remodelling takes place on bone surfaces and consists of four sequential and distinct phases of cellular events, namely: activation, resorption, reversal and formation. Through this process, removal of old bone is coupled in space and time by replacement of new bone. Oestrogen deficiency leads to substantial increases in the number of BMUs and prolongs the resorption phase of the remodelling cycle, leading to a net loss of bone. Despite a consequent net increase in bone formation, this is insufficient to compensate for enhanced bone resorption due to increased osteoblast apoptosis, which is also induced by oestrogen deficiency[79].

Based on studies in ovariectomised rodents, several pro-inflammatory cytokines have been postulated to mediate the effects of oestrogen on bone resorption, with tumour-necrosis

factor (TNF)- α and interleukin (IL)-1β identified as drivers of osteoclastogenesis[79,80]. In addition, there is evidence to suggest that oestrogen deficiency increases the production of receptor activator of nuclear factor κB ligand (RANKL) by cells in the bone microenvironment, leading to increased bone resorption[81]. Sclerostin, a glycoprotein predominantly expressed by osteocytes, inhibits bone formation by inhibiting osteoblast differentiation[82]. Several human studies have demonstrated that serum sclerostin decreases in the face of osterogen deficiency, and these findings suggest that sclerostin may be a pathway by which oestrogen modulates bone remodelling[78].

The interactions between the bone and reproductive systems in the context of T1D are unique and complex. The hallmark of T1D is insulin deficiency, where the lack of insulin signalling disrupts bone metabolism and sex steroid production. T1D is associated with low bone mass and disturbed bone microarchitecture, which increases the propensity for fracture. The absence of insulin is also implicated in aforementioned perturbations of the HPO axis, leading to HH and oestrogen deficiency, which has further deleterious effects on bone. On the other hand, the widespread use of intensive insulin therapy as standard practice in the management of T1D has led to further repercussions on bone and reproductive health. Insulin use is associated with a three-fold increased risk of falls in women with diabetes, compared with non-diabetic controls[83]. The risk of hypoglycaemia is also increased with insulin therapy, which portends an increased fracture risk in T1D[84]. Several large cohort studies have suggested an association between T1D and earlier menopause. This phenomenon may be secondary to glucose toxicity from poor metabolic control, or related to accelerated depletion of ovarian follicular stores with intensive insulin therapy[49]. The reproductive lifespan, or duration of lifetime oestrogen exposure, may be reduced in women with T1D, ultimately increasing the risk of osteoporosis and fractures. Overall, the role of insulin is integral in the pathways of both reproductive and skeletal dysfunction. The effect of T1D on skeletal fragility may be in part mediated by oestrogen deficiency, although further studies are needed to confirm this relationship.

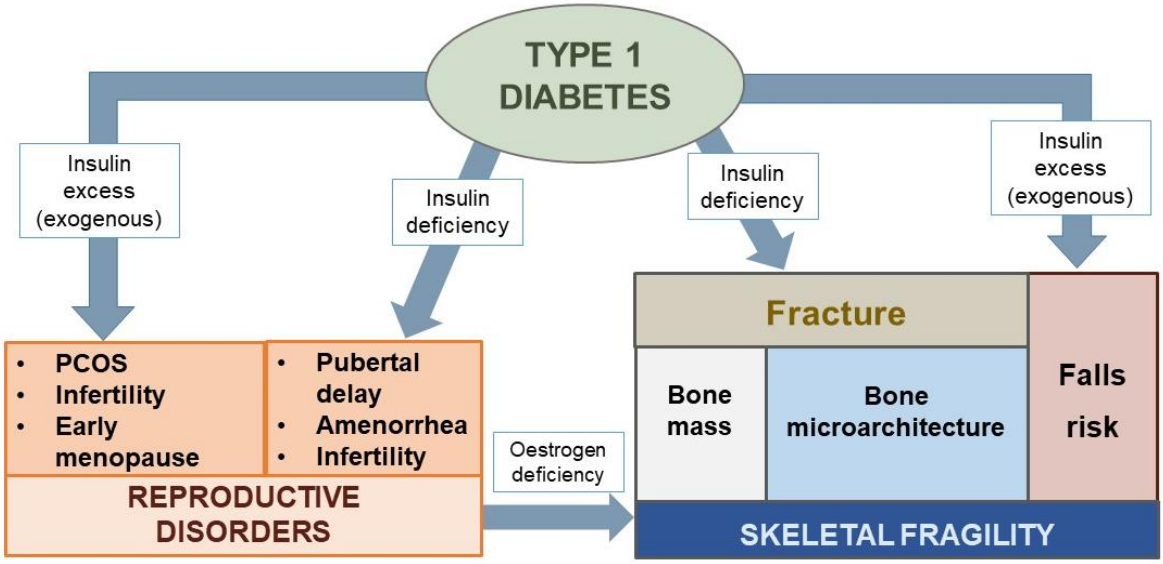

Figure 2. Interactions between bone and reproductive disorders in T1D.

1.4 Summary and Research Aims

Individuals with T1D have an increased risk of skeletal fragility and reproductive disorders. The mechanisms by which T1D affects the bone and reproductive system are diverse and not completely understood. The fundamental objectives of this research are to gain a better understanding of bone and reproductive sequelae as under-recognised metabolic complications of T1D, and to promote greater awareness amongst clinicians, in order to optimise opportunities for screening and prevention in high-risk individuals.

Overall, the body of work presented in this thesis aims to:

1. Evaluate fracture risk in young to middle-aged adults with T1D;
2. Examine the gaps in mechanisms and clinical risk factors associated with fracture in T1D;
3. Summarise the history, spectrum and aetiologies of female reproductive disorders across the lifespan in T1D;

4. Assess the contemporary risk of menstrual and reproductive disorders in young women with T1D;
5. Evaluate potential interactions between bone and reproductive health in the unique context of T1D.

2. CHAPTER 2: BONE HEALTH IN TYPE 1 DIABETES

2.0 MECHANISMS OF SKELETAL FRAGILITY IN T1D

2.0.1 Bone mineral density in T1D

Compared with their non-diabetic counterparts, individuals with T1D appear to have a lower BMD at both the lumbar spine and femoral neck. Vestergaard[37] reported -0.22 and -0.38 reduction in BMD Z-scores at the lumbar spine and hip in those with T1D, compared with controls. These findings were also reproducible in a later meta-analysis by Shah and colleagues, who observed that femoral neck BMD was decreased by 0.055 g/cm^2, in individuals with T1D. Importantly, decreased BMD affects both males and females with T1D[36].

Insulin has anabolic actions on bone *in vitro*. Deficiencies in insulin and insulin-like growth factor (IGF-1), as seen in individuals with T1D, have been postulated to impair bone formation. Murine models of T1D exhibit substantial bone loss and impaired bone strength, attributable to reduced bone formation and increased bone resorption[85]. In humans with T1D, low bone formation has been confirmed, although bone resorption appears to be decreased or unchanged. In children and young adults, low bone turnover markers, procollagen type 1 N propeptide (P1NP) and C-telopeptide (CTX) have been described[86,87], consistent with a state of low bone turnover. Hyperglycaemia is known to suppress osteoblast differentiation and signalling, and may potentially impair bone formation. Various AGEs and their receptors have been implicated in the development of diabetic microvascular complications[85]. These AGEs can also play a role in disrupting the collagen matrix of bone, thereby compromising bone biomechanical properties.

Peak bone mass is acquired at puberty and accrued by the third decade of life, after which changes in BMD are minimal, with the exception of menopause. The diagnosis of T1D in

early life may interfere with peak bone mass accrual, leading to reduced BMD in adulthood. Several other mechanisms that decrease BMD in this cohort include increased urinary calcium excretion (hypercalciuria), leading to a negative calcium balance, and alterations in vitamin D metabolism, particularly in those with diabetic nephropathy and/or chronic kidney disease (CKD)[37]. In addition, hypogonadism and concomitant autoimmune diseases, such as coeliac disease and autoimmune thyroid disease, nutritional deprivation in the face of poor metabolic control, can further exacerbate the reduction in BMD.

2.0.2 Bone structure and microarchitecture in T1D

Beyond BMD and bone mineral content, the mechanical competence and integrity of bone are in part dependent on bone structure and microarchitecture. Fracture risk assessment using BMD alone disregards other important features of bone's biomechanical competence, such as bone geometry, cortical thickness and trabecular microarchitecture[88]. Indeed, the modest reductions in BMD does not adequately explain the significantly increased risk of fracture in T1D. Histomorphometric analysis is the gold standard for the quantitative assessment of bone structure, bone modelling and remodelling[89]; however, this process is invasive, and studies assessing bone histology in persons with T1D are limited. Newer imaging modalities, such as HR-pQCT and TBS, have allowed for the indirect quantification of bone microarchitecture and bone strength to be carried out non-invasively. TBS, a gray-level textural metric extracted from lumbar spine DXA images, is correlated with bone microarchitecture, and has been shown to have predictive value for fracture independent of FRAX®[90]. Deficits in trabecular microarchitecture have been reported at the ultra-distal radius and tibia using HR-pQCT, in young adults with T1D and microvascular disease, compared with age- and sex-matched controls[91]. Amongst pre-menopausal women with T1D, deficits in trabecular bone microarchitecture have been correlated with lower levels of IGF-1[86]. Two studies utilising TBS to evaluate trabecular bone quality at the lumbar spine in adults with T1D have shown that lower TBS scores are associated with components of the

metabolic syndrome, insulin resistance[92], and prevalent fractures[93]. In adolescents with T1D, bone sizes were significantly smaller compared with controls[94,95], which could translate to reduced bone strength to resist fractures. Therefore, parameters of bone microarchitecture, such as TBS, may be a useful adjunct to BMD when assessing fracture risk in patients with T1D.

2.0.3 Recurrent vertebral fractures in a young adult: a closer look at bone health in type 1 diabetes mellitus (*Endocrinology, Diabetes & Metabolism Case Reports*)

Endocrinology, Diabetes & Metabolism CASE REPORTS

E P Thong and others | Bone health in type 1 diabetes mellitus

ID: 18-0010; May 2018
DOI: 10.1530/EDM-18-0010

Recurrent vertebral fractures in a young adult: a closer look at bone health in type 1 diabetes mellitus

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Summary

The association between type 1 diabetes mellitus (T1DM) and bone health has garnered interest over the years. Fracture risk is known to be increased in individuals with T1DM, although bone health assessment is not often performed in the clinical setting. We describe the case of a 21-year-old male with longstanding T1DM with multilevel vertebral fractures on imaging, after presenting with acute back pain without apparent trauma. Dual-energy X-ray absorptiometry (DXA) revealed significantly reduced bone mineral density at the lumbar spine and femoral neck. Extensive investigations for other secondary or genetic causes of osteoporosis were unremarkable, apart from moderate vitamin D deficiency. High-resolution peripheral quantitative computed tomography and bone biospy revealed significant alterations of trabecular bone microarchitecture. It later transpired that the patient had sustained vertebral fractures secondary to unrecognised nocturnal hypoglycaemic seizures. Intravenous zoledronic acid was administered for secondary fracture prevention. Despite anti-resorptive therapy, the patient sustained a new vertebral fracture after experiencing another hypoglycaemic seizure in his sleep. Bone health in T1DM is complex and not well understood. There are significant challenges in the assessment and management of osteoporosis in T1DM, particularly in young adults, where fracture prediction tools have not been validated. Clinicians should be aware of hypoglycaemia as a significant risk factor for fracture in patients with T1DM.

Learning points:

- Type 1 diabetes mellitus (T1DM) is a secondary cause of osteoporosis, characterised by reduced bone mass and disturbed bone microarchitecture.
- Hypoglycaemic seizures generate sufficient compression forces along the thoracic column and can cause fractures in individuals with compromised bone quality.
- Unrecognised hypoglycaemic seizures should be considered in patients with T1DM presenting with fractures without a history of trauma.
- Patients with T1DM have increased fracture risk and risk factors should be addressed. Evaluation of bone microarchitecture may provide further insights into mechanisms of fracture in T1DM.
- Further research is needed to guide the optimal screening and management of bone health in patients with T1DM.

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Background

Type 1 diabetes mellitus (T1DM) is an immune-mediated condition culminating in the destruction of pancreatic beta cells, which are necessary for insulin production. T1DM is commonly diagnosed in childhood and young adults, thus reflecting the burden of this chronic disease in a young population, who are ultimately at risk of the long-term complications of diabetes. In recent years, there has been growing interest and awareness of the pathophysiology and mechanisms behind diabetic bone disease. Although T1DM is an established risk factor for osteoporosis and fracture, bone health in patients with T1DM is not routinely assessed. This may be in part due to the lack of guidelines for fracture risk assessment and management of such patients, particularly in children and young adults. We present the challenges in diagnosis and management of recurrent fractures secondary to hypoglycaemic seizures in a patient with T1DM.

Case presentation

A 21-year-old male Caucasian university student presented to the emergency department after awaking with sudden-onset, severe inter-scapular back pain. Multiple thoracic vertebral fractures were initially appreciated on plain X-ray films, and subsequent magnetic resonance imaging (MRI) confirmed widespread acute and subacute compression fractures throughout the thoracic and lumbar spine at T2–5, T9 and T10, L4 and L5 (Fig. 1). The patient reported no antecedent trauma or recent falls.

His history was significant for a 12-year duration of T1DM, which was managed with a basal-bolus insulin regimen, under the care of an endocrinologist. He reported good glycaemic control historically and self-monitoring of blood sugar levels several times a day with infrequent hypoglycaemic episodes. He was a non-smoker and only consumed alcohol socially. Dietary calcium intake was adequate, averaging three servings daily. There was no history of fractures in childhood, and no personal or

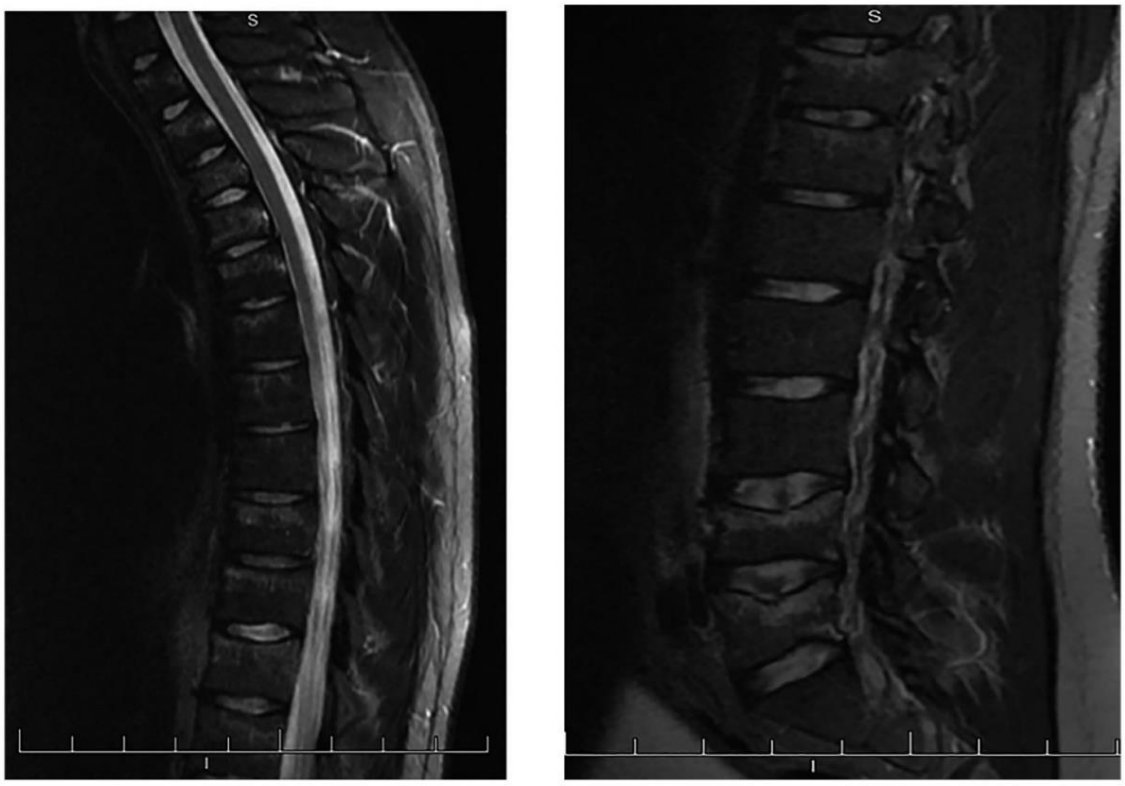

Figure 1
T2-sagittal MRI demonstrating hyperintense marrow signal abnormalities in several vertebral compression fractures. MRI, magnetic resonance imaging.

family history of bone disease or minimal trauma fractures. Apart from multiple daily injections of insulin detemir and aspart, he reported no other regular medications or previous glucocorticoid use. Clinical examination revealed a well-virilised male with normal testicular volumes, and a height and weight of 181 cm and 94 kg respectively. Fundoscopy revealed no evidence of retinopathy, and neurological examination was unremarkable; there was no evidence of blue sclerae or kyphosis.

Investigation

Initial laboratory investigations revealed a normal electrolyte profile and renal function, full blood count and thyroid function tests. He had a glycated haemoglobin of 7.8% (62 mmol/L) and had no evidence of urinary microalbuminuria. Moderate vitamin D deficiency (28 nmol/L) was identified; however, corrected calcium, phosphate and parathyroid hormone levels were all within reference limits. Coeliac disease antibodies were negative, as were other investigations for secondary osteoporosis, including Cushing's syndrome, hyperthyroidism, hypogonadism, liver disease, mastocystosis, idiopathic hypercalciuria and multiple myeloma (Table 1). Bone-turnover markers (C-terminal telopeptide (CTX) and procollagen type 1 amino-terminal propeptide (P1NP)) were elevated, in keeping with the increased bone-turnover state in the context of his recent fractures. Bone mineral density on DXA was severely reduced, with *Z*-scores of −2.9 at L2–L3 and −3.0 at the left femoral neck respectively.

Four months later, the patient developed new acute fractures at T7 and L2, after a witnessed hypoglycaemic seizure at home. During his inpatient admission, he was noted to have multiple nocturnal hypoglycaemic episodes with impaired awareness of hypoglycaemia. Investigations for a primary seizure disorder with MRI brain and electroencephalogram were unremarkable.

Given the extensive fracture history, a bone marrow aspirate and trephine (with tetracycline-labelled bone biopsy) of the posterior iliac crest was performed to exclude a malignant cause. No haematological or malignant disease was identified, but disruption to the bone trabecular architecture was evident in cancellous bone, characterised by a reduction in trabecular number, thinning and increased separation of trabeculae (Fig. 2). There were no features of osteomalacia, indicated by normal tetracycline uptake at the bone-mineralising surfaces. High-resolution peripheral quantitative computed tomography (HR-pQCT) was also performed of the radius and tibia, revealing significant cortical and trabecular deficits. In particular, his cortical and trabecular volumetric bone mineral density, and cortical thickness, were under the 10th centile of age-, sex- and site-matched

Table 1 Summary of biochemical investigations and HR-pQCT parameters.

	Value	RR or centile*
Biochemical investigations		
HbA1c (%)	**7.8%**	≤6.0
Creatinine (μmol/L)	96	60–105
Urea (mmol/L)	4.4	4.4–9.0
Estimated GFR (mL/min/1.73 m²)	>90	>90
Alkaline phosphatase (U/L)	136	42–135
Corrected calcium (mmol/L)	2.49	2.14–2.50
Magnesium (mmol/L)	0.90	0.66–1.07
Phosphate (mmol/L)	0.65	0.60–1.30
Parathyroid hormone (pmol/L)	4.5	1.6–6.9
Vitamin D (nmol/L)	**28**	>50
TSH (mU/L)	1.60	0.3–5.00
FSH (U/L)	2.8	1.4–13.6
Luteinising hormone (U/L)	1.0	0.6–12.1
Total testosterone (nmol/L)	20.6	8.0–30.0
Prolactin (U/L)	218	73–306
Cortisol, post ODST (nmol/L)	42	<50
Transglutaminase AB (U/mL)	0	0–4
Gliadin IgA (units)	5	0–19
Gliadin IgG (units)	2	0–19
Tryptase (μg/L)	4.1	0.0–11.4
Serum protein electrophoresis	NPD	
CTX (ng/L)	**1435**	400–900
P1NP (μg/L)	**151**	15–115
HR-pQCT) parameters		
Radius		
Total BMD (mg/HA/cm³)	**274.2**	<10th
Cortical BMD (mg/HA/cm³)	**792.3**	<10th
Trabecular BMD (mg/HA/cm³)	177.0	<25th
Trabecular number (1/mm)	**1.67**	<10th
Trabecular thickness (mm)	0.088	75th
Trabecular separation (mm)	0.511	90th
Cortical thickness (mm)	**0.62**	<10th
Tibia		
Total BMD (mg/HA/cm³)	**246.3**	<10th
Cortical BMD (mg/HA/cm³)	**835.0**	<10th
Trabecular BMD (mg/HA/cm³)	**172.1**	<10th
Trabecular number (1/mm)	1.87	25th
Trabecular thickness (mm)	**0.077**	10th
Trabecular separation (mm)	0.459	75th
Cortical thickness (mm)	**0.82**	<10th

Abnormal values are denoted in bold.
*RR is presented for biochemical investigations and centiles for HR-pQCT parameters. Centiles are derived from age-, sex- and site-specific centile curves for HR-pQCT parameters using a Canadian reference population from the study by Burt *et al.* (1).
BMD, bone mineral density; CTX, C-terminal telopeptide; GFR, glomerular filtration rate; HA, hydroxyapatite; HR-pQCT, high-resolution peripheral quantitative computed tomography; NPD, no paraprotein detected; ODST, overnight dexamethasone suppression test; P1NP, N-terminal propeptide of type 1 collagen; RR, reference range; HbA1c, glycated haemoglobin; TSH, thyroid stimulating hormone; FSH, follicle stimulating hormone; AB, antibodies.

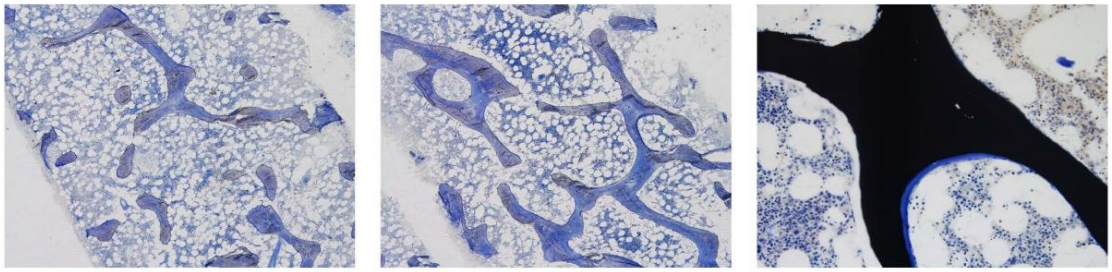

Figure 2
(A) Toluidine blue stain at ×40 showing reduced numbers, abnormal shapes, thinning and reduced connectivity of the bony trabeculae. (B) Toluidine blue stain at ×40 showing an area of relatively normal trabecular bone formation in the patient. (C) Von Kossa stain for calcium at ×100 showing a normal small smooth segment of osteoid on the surface of normally calcified bone.

normative values derived from a reference population of adults (1) (Table 1). A clinical geneticist reviewed the patient, and no hereditary cause of bone disease was identified. Therefore, the working diagnosis was extensive vertebral fractures secondary to unrecognised hypoglycaemic seizures, on a background of impaired bone microarchitecture.

Treatment

His initial management consisted of non-opioid analgesia, vitamin D replacement and insulin optimisation to prevent further episodes of hypoglycaemia. Zoledronic acid (5 mg) was administered intravenously for fracture prevention after he was vitamin D replete.

Outcome and follow-up

Fourteen months later, the patient re-presented with back pain, following another hypoglycaemic seizure, which occurred in his sleep. Repeat spine imaging revealed a new fracture of the L1 vertebra. Given the recurrent fracture despite anti-resorptive therapy, consideration was given to teriparatide, an anabolic agent, for the treatment of his severe osteoporosis. Continuous glucose monitoring was also organised to better delineate his nocturnal glycaemic pattern, in a bid to reduce further hypoglycaemic episodes.

Discussion

The link between bone health and T1DM has garnered increasing attention in recent years. Meta-analyses of observational studies have reported a threefold to sevenfold increased risk of hip fracture in individuals with T1DM, compared to controls (2, 3). T1DM is an established cause of secondary osteoporosis and the pathogenesis of fracture in this population appears to be multifactorial (Table 2). T1DM is characterised by a state of hypoinsulinaemia and insulin-like growth factor (IGF-1) deficiency. This has been postulated to impair osteoblast function, giving rise to a low bone-turnover state (4, 5, 6). Peak bone mass is acquired at puberty and accrued by the third decade of life. The development of T1DM in childhood may interfere with peak bone mass accrual, such that individuals may have reduced bone mineral density (BMD) in adulthood. Advanced glycation end products (AGEs) from chronic hyperglycaemia can

Table 2 Mechanisms of fracture in individuals with type 1 diabetes mellitus.

Reduced bone mass
• Insulin/IGF-1 deficiency
• Hypogonadism
• Nephropathy (CKD-MBD)
• Concomitant autoimmunity: Graves' and coeliac disease
• Failure to achieve peak bone mass (childhood-onset T1DM)
Impaired bone quality and biomechanical properties
• Disrupted trabecular bone architecture
• Altered cortical bone geometry
• Hyperglycaemia: altered bone collagen matrix by AGEs
Increased risk of falls and trauma
• Neuropathy
• Retinopathy
• Hypoglycaemia
• Amputations
Other
• Vitamin D deficiency
• Chronic inflammation
• Family history of osteoporosis
• Smoking
• Genetic bone disorder, e.g. OI
• Corticosteroid use

AGEs, advanced glycation end products; CKD-MBD, chronic kidney disease mineral and bone disorder; IGF-1, insulin-like growth factor; OI, osteogenesis imperfecta.

disrupt collagen matrix and impact negatively on bone biomechanical properties (5, 6, 7). Altered bone mineral metabolism is often seen with diabetic chronic kidney disease, and concomitant autoimmunity, such as thyroid and coeliac disease, may contribute to further loss of bone mass. Finally, the long-term sequelae of diabetes, including retinopathy, neuropathy and hypoglycaemia may contribute to reduced physical function and increased propensity to falls (4).

Hypoglycaemic seizures, such as in our patient, have been implicated in multilevel vertebral fractures in a few case reports (8, 9, 10). Compressive forces along the anterior and middle columns of the mid-thoracic kyphotic curve during seizure activity can lead to a unique dispersion of 'flexion fractures' in the upper to mid-thoracic vertebrae (8). These fractures are usually not clinically apparent; however, the reported prevalence of seizure-associated asymptomatic fracture is as high as 15–16% (8, 9).

Clinical risk factors associated with fracture in patients with T1DM have not been well defined, although several studies have reported a consistent association of microvascular complications with increased fracture risk (5). Although BMD is typically used to predict fracture risk in the general population, it may underestimate fracture risk in patients with T1DM, especially in young adults in whom the use of fracture prediction tools has not been validated (11). Bone microarchitecture is an important determinant of bone quality and strength. Histomorphometric analysis is the gold standard for the evaluation of tissue-level bone activity (12); however, it is invasive and studies examining bone histology in humans with T1DM are limited. Novel imaging modalities such as trabecular bone score and HR-pQCT can provide indirect assessments of bone microarchitecture, which may be a useful adjunct to DXA, in the assessment of bone health in patients with T1DM.

Shanbhougue *et al.* (13) demonstrated deficits in HR-pQCT-derived trabecular microarchitecture parameters at the ultradistal radius and tibia in adults with T1DM and microvascular disease, compared to age- and gender-matched non-diabetic controls. Our patient exhibited a similar pattern of bone microarchitecture disturbance on HR-pQCT, and bone histomorphometric findings were consistent with that observed in murine models of T1DM (14, 15).

This case study demonstrates recurrent fractures secondary to trauma from hypoglycaemic seizures underpinned by impaired bone microarchitecture, in a young adult with T1DM, with no other secondary causes of accelerated bone loss. While our patient was young, exhibited reasonable glycaemic control and had no microvascular complications, we postulate that the development of T1DM in early childhood may have had significant detrimental impacts on peak bone mass accrual and bone microstructure. To our knowledge, this is the first report correlating HR-pQCT and histomorphometric evidence of dysfunctional bone microarchitecture in a fracturing patient with T1DM.

Despite treatment with intravenous bisphosphonate, the patient sustained a new vertebral fracture after another hypoglycaemic seizure. This raises the importance of hypoglycaemia prevention in T1DM, particularly those with established osteoporosis. Recurrent hypoglycaemic episodes can diminish the sympathoadrenal response and lower the threshold at which symptoms occur (16). Patients with impaired awareness of hypoglycaemia are at risk of falls and trauma, and plain radiographs of the thoracolumbar spine should be considered to exclude occult fractures in high-risk patients, where appropriate. The use of continuous glucose monitoring sensors may aid in the detection of unrecognised nocturnal hypoglycaemia.

The optimal therapy for low BMD in young adults with T1DM has not been evaluated. Although *post hoc* analyses of randomised controlled trials have demonstrated similar efficacy of bisphosphonates in incrementing BMD in post-menopausal patients with type 2 diabetes mellitus and non-diabetic controls (17), this may not be applicable to a younger cohort with T1DM. Given that bone disease in T1DM is characterised by a low bone-turnover state, treatment with bisphosphonates may further suppress bone turnover in such patients. Anabolic agents, such as teriparatide, increase bone formation and may be an ideal therapy for patients with T1DM bone disease. Intermittent parathyroid hormone (PTH) therapy has been shown to increase trabecular bone volume in rodents with T1DM (18), but no such studies have been performed in humans thus far.

In conclusion, T1DM causes disruption to bone microarchitecture and matrix in several ways and can increase susceptibility to falls and trauma, from hypoglycaemia or microvascular complications (Table 2). Unrecognised hypoglycaemic seizures should be considered in patients with T1DM presenting with fractures without a history of trauma. The management of osteoporosis in a young adult with T1DM is challenging, and further research is needed to guide the optimal screening and management of bone health in T1DM.

Declaration of interest
The authors declare no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Patient consent
Written informed consent for publication of their de-identified clinical information and clinical images was obtained from the patient.

Author contribution statement
E P T performed the literature review and wrote the manuscript; E P T, S C and F M were involved in the care of the patient; J F was the anatomical pathologist and P W, P J F and H T made critical revisions. All authors approved the final version of the manuscript.

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Received in final form 27March2018
Accepted 19April2018

2.1 CLINICAL RISK FACTORS FOR FRACTURE IN T1D

Clinical characteristics of increased skeletal fragility are important to define, in order to identify those at greatest risk of fracture. In general, older age and female sex are universally recognised as important determinants of fracture risk. However, this may not necessarily be the case in the T1D cohort, where the relative risk of hip fractures appear to be elevated in younger subjects, in both men and women alike[33,96]. Although the meta-analysis by Vestergaard[37] did demonstrate clear associations between glycaemic control and fracture risk, recent studies suggest that poorer glycaemic control is implicated in increased fracture tendencies. In a large UK case-control study, an HbA1c greater than 8.0% was found to confer a 40% higher risk of fracture compared with an HbA1c at 7.0% or under[97]. A smaller cross-sectional study of men and premenopausal women with T1D reported an independent association of long-term glycaemic control with prevalent fractures, where fracture risk doubled for every 1-SD increased in median HbA1c[98]. Increased serum levels of pentosidine, an AGE and by-product of chronic hyperglycaemia, has been reported in individuals with T1D and prevalent fractures, compared with those without fracture[99].

The presence of microvascular complications is associated with increased fracture risk in individuals with T1D. A Swedish population-based historical cohort study examining the relationship between T1D and hip fractures found that the standard hospitalisation ratio for hip fracture was increased by 17 to 33-fold in those with T1D and microvascular complications, compared with 3 to 6-fold in those with T1D alone, with non-diabetic controls as the reference group[100]. A large Danish register-based case-control study observed that fracture risk was increased by 2.4-, 3.4- and 2.8-fold for those with retinopathy, nephropathy and neuropathy, while patients without complications had a 1.6-fold increased risk of fracture, compared with non-diabetic controls. Notably, only diabetic kidney disease was an independent risk factor, in addition to T1D[101]. Both aforementioned studies lacked information regarding glycaemic control, raising the possibility of poor metabolic control as a

confounder in the association between fracture and microvascular complications[102]. In addition, neuropathy and retinopathy are associated with reduced balance and may contribute to a higher falls risk in affected individuals. Furthermore, diabetic microvascular complications do not arise until at least ten years from diagnosis of diabetes, and may be a proxy for longer duration of T1D. The effect of diabetes duration, independent of microvascular complications, is unclear. Remarkably, a study examining bone health in individuals with T1D for over 50 years[103], observed that there was a low prevalence of hip and wrist fractures in this cohort, with BMD in the age-matched normal range at various skeletal sites. Indices of the metabolic syndrome and cardiovascular disease, but not HbA1c, was associated with lower BMD at the femoral neck, suggesting that metabolic factors outside of glycaemic control may play a role in fracture pathophysiology.

The association between T1D and other autoimmune diseases is well described in the literature. Coeliac disease and Graves' disease are established causes of secondary osteoporosis, and could exacerbate the fracture risk in individuals with T1D. A cross-sectional study of children and adults, showed that those with concomitant coeliac autoimmunity and T1D exhibited significantly lower BMD compared with age-, sex- and BMI-matched T1D controls[104]. Few studies have evaluated the impact of concomitant T1D and autoimmune disease on fracture risk. However, coeliac disease was found to increase the risk of fractures in females, but not males with T1D, in the largest cohort study of individuals with T1D and fracture outcomes to date[33].

Hypoglycaemia is an undesirable side effect of insulin therapy, which is associated with considerable morbidity, psychological distress[105] and increased falls risk[106]. Only one study has evaluated the impact of hypoglycaemia on fracture risk in T1D. Jensen and Vestergaard[84] reported a 58% increased risk of fracture with hypoglycaemia, which is consistent with findings from an earlier study of hypoglycaemia and fracture risk in T2D[107]. Importantly, one in six individuals with T1D from this study experienced severe

hypoglycaemia resulting in hospital admissions, demonstrating that prevention of hypoglycaemia should be a priority in fracture prevention. The proposed mechanisms and risk factors for fracture in individuals with T1D are summarised below in Table 3.

Proposed mechanisms and risk factors for skeletal fragility in T1D	
Low bone mass/↓ bone turnover • Insulin/IGF-1 deficiency • ↓ peak bone mass accrual • Nephropathy (chronic kidney disease-mineral bone disease)	**Impaired bone microarchitecture/strength** • ↑ trabecular thinning and separation • ↑ cortical porosity • Altered bone geometry
Hyperglycaemia • Disruption of bone collagen matrix by AGEs • Hypogonadism (due to perturbations of HPO-axis) • Microvascular disease	**Concomitant autoimmune disease** • Coeliac disease • Hyperthyroidism • Addison's disease
Increased falls risk • Neuropathy • Retinopathy/vision impairment • Hypoglycaemia • Amputations • Arthritis	**Other** • Other co-existent secondary causes of osteoporosis, including rheumatoid arthritis, liver disease, inflammatory conditions etc. • Vitamin D deficiency • Smoking • Medications, including glucocorticoids, antiepileptic drugs etc. • Family history of osteoporosis

Table 3. Proposed mechanisms and risk factors for skeletal fragility in T1D

2.1.1 Increased prevalence of fracture and hypoglycaemia in young adults with concomitant type 1 diabetes mellitus and coeliac disease (*Clinical Endocrinology*)

Received: 5 July 2017 | Revised: 31 August 2017 | Accepted: 25 September 2017

DOI: 10.1111/cen.13488

ORIGINAL ARTICLE

WILEY

Increased prevalence of fracture and hypoglycaemia in young adults with concomitant type 1 diabetes mellitus and coeliac disease

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Funding information
This study was not funded. EPT is supported by an Australian Research Training Program (RTP) postgraduate scholarship. PW and FM are supported by an Osteoporosis Australia/ Australia and New Zealand Bone and Mineral Society Clinical Grant. HT is an NHMRC Practitioner Fellow.

Summary

Background: Both Type 1 diabetes mellitus (T1DM) and coeliac disease (CD) are independently associated with reduced bone mineral density (BMD) and increased fracture risk. Whilst poorer glycaemic control and increased microvascular complications have been described, the literature examining bone health and fractures in adults with concomitant T1DM and CD (T1DM + CD) is limited.

Objective: To evaluate fracture prevalence and explore associations with glycaemic control, hypoglycaemia and microvascular disease in T1DM + CD compared with T1DM alone.

Methods: We conducted a retrospective cross-sectional study of young adults with T1DM, who attended diabetes clinics at a large tertiary referral centre between August 2016 and February 2017. Clinical information, radiological and biochemistry results were extracted from medical records. Patients with comorbid chronic kidney disease, glucocorticoid use, hypogonadism and untreated hyperthyroidism were excluded.

Results: A total of 346 patients with T1DM alone (median age 23 years) and 49 patients with T1DM + CD (median age 24 years) were included. Median age, gender distribution, BMI, haemoglobin A1c, daily insulin dose and serum 25-hydroxyvitamin D levels were similar between groups. Higher adjusted fracture risk was observed in T1DM + CD compared with T1DM (12.2% vs 3.5%; OR 3.50, 95% CI 1.01-12.12, $P = .01$), yet BMD was only measured in 6% of patients. The adjusted risk of hypoglycaemia ≥2/week was greater for T1DM + CD (55% vs 38%, OR 3.28, 95% CI 1.61-6.69, $P = .001$); however, this was not independently associated with fractures. Replete vitamin D (≥ 50 nmol/L) was associated with less hypoglycaemia (OR 0.48, 95% CI 0.29-0.80; $P = .005$), but not with fractures.

Conclusions: Coeliac disease status was independently associated with increased fracture prevalence in young adults with T1DM. Recurrent hypoglycaemia was also increased in T1DM + CD, although hypoglycaemia was not independently associated with fractures. Prospective studies are required to determine the long-term impacts of CD on bone health and glycaemic control in patients with T1DM.

1 | INTRODUCTION

The association between Type 1 diabetes mellitus (T1DM) and coeliac disease (CD) is well described. Coeliac disease is an autoimmune condition characterized by gluten-induced enteropathy, with a global prevalence of 3%-16% in individuals with T1DM.[1,2] Both these autoimmune conditions share similar genetic and environmental risk factors,[3] which may explain the 4-6 fold increased prevalence of CD in T1DM, compared with the general population.[4] The diagnosis of CD is made by serological tests and confirmed on duodenal biopsy.[5] The hallmark of this condition is duodenal villous atrophy and intraepithelial lymphocytosis, which may give rise to nutrient deficiencies and gastrointestinal symptoms. However, not all cases present with overt symptoms, and children with T1DM are more likely to have subclinical disease,[6] potentially manifesting as increased glycaemic variability and hypoglycaemia, growth failure and decreased bone mass.[6-8]

An increased risk of microvascular complications[9,10] has been reported in patients with concomitant T1DM and coeliac disease (T1DM + CD). The mechanism by which CD may impact on microvascular disease in T1DM is unclear. The causes are likely to be multifactorial and include chronic inflammation, nutritional deficiencies and endothelial dysfunction.[9] CD is also a recognized risk factor for secondary osteoporosis and fracture.[11,12] The main driver of bone loss is thought to be calcium malabsorption and vitamin D deficiency, leading to secondary hyperparathyroidism. Hypogonadotropic hypogonadism is also associated with CD, and this may contribute to further bone loss.[11]

T1DM is associated with impaired bone quality and increased fracture rates. Recent large observational studies and meta-analyses have reported a two- to seven-fold greater risk of hip fracture in patients with T1DM compared with nondiabetic controls.[13-15] Osteoblast dysfunction, disruption of collagen cross-linking, altered bone material properties calcium and vitamin D deficiencies have been postulated as mechanisms for skeletal fragility in T1DM.[16] The risk of impaired bone microarchitecture and fracture appear to be greater in those with poor glycaemic control and microvascular complications.[17,18]

As T1DM and CD are both independently associated with bone fragility and fracture risk, this may be additive in T1DM + CD. Few studies have evaluated bone health in those with T1DM + CD, with some studies suggesting a negative impact on bone mineral density (BMD),[7,19,20] whereas a large cohort study did not demonstrate increased fracture risk.[21] These studies were primarily conducted in children, and extrapolation of results to an adult population is potentially inappropriate. We therefore aimed to investigate the relationship between CD status on fracture rates in young adults with T1DM and explore potential interactions between hypoglycaemia, glycaemic control and microvascular complications with fracture risk.

2 | METHODS

2.1 | Patients

We retrospectively reviewed medical records of patients attending specialist diabetes outpatient clinics between August 2016 and February 2017 at Monash Health, a large tertiary centre and accredited Australian centre of excellence in diabetes. All patients attending with T1DM aged between 18 and 45 years of age were included in the study, except those with comorbid end-stage kidney disease, previous transplantation, glucocorticoid use, malignancy, hypogonadism and untreated hyperthyroidism, who were excluded. A history of hypogonadism was established from the medical record, defined in males as the use of androgen-replacement therapy or low testosterone levels (<8 nmol/L), documented on 2 separate occasions. In females, hypogonadism was defined as the use of hormone-replacement therapy for induction of pubertal development, menopause before the age of 40 years, or documentation of low oestradiol levels (<73 pmol/L), on 2 separate occasions at least 6 months apart. In addition, females with documented amenorrhoea (of greater than 3 months duration) or individuals with eating disorders were excluded from the study.

Clinical information pertaining to previous fracture, duration of T1DM, glycaemic control, height and weight, hypoglycaemia, daily dose of insulin, microvascular complications and CD status were obtained from medical records, radiological and laboratory reports. This study was approved by the Monash Health Human Research Ethics Committee.

2.2 | Type 1 diabetes mellitus and coeliac disease

The diagnosis of T1DM was confirmed on medical records or biochemically on antibody positivity to glutamic acid decarboxylase (GAD) and/or islet antigen-2 (IA-2). All patients with T1DM were screened for CD with deamidated gliadin and tissue transglutaminase (tTG) antibody testing, as part of an annual complication and autoimmune disease screening policy. Patients were classified as having CD based on sero-positivity to coeliac antibodies and/or histological evidence of villous atrophy on small bowel biopsy. Information regarding documented gastrointestinal symptoms and adherence to a gluten-free diet (GFD) in individuals with CD were also obtained.

2.3 | Other autoimmune diseases

One individual in the T1DM + CD group and 3 individuals in the T1DM group had Graves' disease, all of whom were biochemically euthyroid for at least 3 months prior to inclusion into the study. Fifteen individuals had established hypothyroidism (T1DM + CD, n = 3; T1DM only, n = 12) and all were biochemically euthyroid. No individual in the study had Addison's disease. Two individuals from the T1DM group had rheumatoid arthritis, and neither reported glucocorticoid therapy.

2.4 | Laboratory analyses

Biochemical tests were performed in 3 different laboratories, with the exception of whole blood haemoglobin A1c (HbA1c) which was measured by ion-exchange high-performance liquid chromatography via Arkray Adams-A1c HA-8160 (Arkray Inc., Kyoto, Japan) at a single laboratory. HbA1c was reported as percentage of glycated haemoglobin (National Glycohaemoglobin Standardisation Program) and in mmol/mol

(International Federation of Clinical Chemistry). Serum creatinine and urinary albumin-to-creatinine ratio were measured on the Beckman Coulter AU 5800 (Beckman Coulter, Brea, CA, USA), Roche Cobas Integra (Roche Diagnostics Ltd., Basel, Switzerland) and Siemens Advia (Siemens Corp., Tarrytown, NY, USA) platforms, respectively, at each laboratory. Serum 25-hydroxyvitamin D levels were measured using the same Diasorin Liaison XL assay (DiaSorin Inc, Stillwater, MN, USA) at all 3 laboratories. A serum 25-hydroxyvitamin D level of ≥ 50 nmol/L was used as a cut-off for vitamin D sufficiency. Calculation of estimated glomerular filtration rate (eGFR) was based on the Chronic Kidney Disease Epidemiology Collaboration equation.[22] Urinary albumin and antibodies to GAD and IA-2 were detected by an enzyme-linked immunofluorescence assay ELISA. Antibodies to deamidated gliadin and tTG were detected using a Luminex-based assay.

2.5 | Fractures

All prevalent fractures, occurring after the diagnosis of T1DM and/or CD, were verified by radiological reports and medical records. Information on fracture mechanisms, age and site at which the fracture(s) occurred in each patient, were also collected.

2.6 | Hypoglycaemia

Patients' glucose monitoring devices or logbooks were reviewed in clinic by experienced clinicians to evaluate the presence and frequency of hypoglycaemia, defined by a recorded capillary blood glucose concentration of ≤3.9 mmol/L. Recorded hypoglycaemia was noted and we were able to directly verify the frequency of hypoglycaemia in all patients on continuous subcutaneous insulin infusion (CSII) therapy, from blood glucose log printouts in individual medical records. Hypoglycaemic events were considered a frequent occurrence if 2 or more events per week, on average, were documented or captured. Frequency of hypoglycaemia was further stratified into: infrequent, 2-4 episodes per week, 5-9 episodes per week and >9 episodes per week. Severe hypoglycaemia was deemed as an episode associated with impaired consciousness or requiring third-party assistance.[23]

2.7 | Microvascular complications

Microvascular diabetic complications were evaluated at least annually. Documented diagnosis of diabetic retinopathy was confirmed on ophthalmology or optometry reports. The presence of microalbuminuria was determined by an albumin-to-creatinine ratio of greater than 3.5 mg/mmol (30 mg/g),[24] confirmed on at least 2 occasions. Neuropathy was assessed in the clinic by vibration, pressure sensation (10 g monofilament test applied at the distal hallux) or pinprick testing. Examination findings were documented in medical records.

2.8 | Statistical analyses

Results were expressed as median and interquartile ranges for continuous data and as percentages for categorical data. Continuous and categorical variables were compared using the Mann-Whitney *U* test and χ^2 and Fisher's exact test, respectively. Univariable analyses were performed, followed by multivariable regression analyses adjusting for significant and clinically relevant covariates for bone loss. Data were analysed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, NY, USA).

3 | RESULTS

A total of 395 patients with T1DM were captured in the study, of which 346 (median age 23 years, range 20-27) had T1DM only and 49 patients (median age 24.0 years, range 23-28) had T1DM + CD. There were no differences in median age, sex, glycaemic control, serum 25-hydroxyvitamin D levels, body mass index (BMI), total daily dose of insulin between the 2 groups (Table 1). The use of CSII devices was also similar across the 2 groups (18.4% vs 24.0%, *P* = .47). Patients with T1DM + CD had a longer median duration of T1DM (14.0 years vs 11.0 years, *P* = .01). Median duration of CD was 8.0 years (range 3-14). The diagnosis of CD preceded T1DM in 6 patients. Median serum HbA1c values (National Glycohemoglobin Standardisation Program) were similar in both groups (8.3% vs 8.3%, *P* = .57), as was the prevalence of microvascular complications (14.3% vs 12.4%, *P* = .65).

TABLE 1 Baseline characteristics of patients

	T1 + CD (n = 49)	T1DM only (n = 346)	*P* -value
Age, y	24 (23-28)	23 (20-27)	.054
Female sex (%)	25 (51.0)	160 (46.2)	.54
Duration of T1DM, y	14 (7.5-20.5)	10 (6-15)	**.01**
CSII (%)	9 (18.4)	83 (24.0)	.47
NGSP HbA1c, %	8.3 (7.5-9.2)	8.3 (7.6-9.7)	.57
IFCC HbA1c, mmol/mol	67 (58-77)	67 (59-81)	.61
eGFR	90 (0)	90 (0)	.35
Urine ACR	0.90 (1.3)	0.90 (1.5)	.67
25(OH) vitamin D, nmol/L	61 (42-74)	54 (39-67)	.13
BMI, kg/m^2	24.9 (21.3-28.2)	25.5 (22.8-28.5)	.21
Total daily insulin dose, IU	58 (34-60)	60 (47-80)	.10
Duration of CD, y	8 (3-14)		
Compliance to GFD (%)	36 (73.5)		

CSII, continuous subcutaneous insulin infusion; NGSP, National Glycohemoglobin Standardisation Program; IFCC, International Federation of Clinical Chemistry; HbA1c, haemoglobin A1c; eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio; BMI, body mass index; CD, coeliac disease; GFD, gluten-free diet.
Interquartile ranges are expressed in brackets. Bold values denote significant P-values (P<0.05).

3.1 | Fractures

A total of 21 fractures were documented in 18 patients. Fracture mechanisms were cross-referenced with emergency department records. In the T1DM + CD group, 2 of 6 patients (33.3%) sustained minimal trauma fractures (MTF), defined as a fracture sustained due to low impact or a fall from standing height or less. Two fractures in this group (33.3%) occurred during sport or motor vehicle accidents. 3 of 12 patients (25%) in the T1DM only group had MTF. Seven fractures in this group (58.3%) were sport- or motor vehicle accident-related. The mechanism of fracture for 4 patients (2 in each group) was unable to be determined. Median age at fracture was 18 and 21 years in the T1 + CD and T1DM alone groups, respectively, with the highest proportion of fractures occurring at the radius and tibia/fibula (Table 2).

TABLE 2 Fracture distribution and mechanisms

	T1 + CD (n = 49)	T1DM only (n = 346)	*P*-value
Number of patients with fracture (%)	6 (12.2%)	12 (3.5%)	**.02**
Female sex	2 (33.3%)	4 (33.3%)	1.00
Median age at fracture, y	18 (12-25)	21 (17-22)	.62
Fracture mechanism			
MTF	2 (33.3%)	3 (25%)	
Sport or trauma related	2 (33.3%)	7 (58.3%)	
Indeterminate	2 (33.3%)	2 (16.7%)	
Fracture site			
Radius	3 (50%)	4 (33.3%)	
Tibia/fibula	1 (16.7%)	4 (33.3%)	
Vertebra	0	2 (16.7%)	
Rib	0	1 (8.3%)	
Foot	1 (16.7%)	1 (8.3%)	
Other	1 (16.7%)	1 (8.3%)	

MTF, minimal trauma fracture. Bold values denote significant P-values (P<0.05).

Overall, a higher proportion of patients had documented fractures in the T1DM + CD group, compared with the T1DM only group (12.2% vs 3.5%, *P* = .02). However, there was no difference in MTF prevalence between the 2 groups (33.3% vs 25.0%, *P* = 1.00). Despite the increased risk of malabsorption and fracture in the T1DM + CD group, only 3 patients (6.1%) had documented evaluation of their BMD within the last 5 years. Information regarding dietary calcium intake was not consistently assessed in patients with T1DM + CD and T1DM alone. No patients had documented calcium supplementation. There were no significant differences between the proportion of patients who were vitamin D sufficient (36.6% vs 42.1%, *P* = .61), and who received supplementation with vitamin D (14.3% vs 18.2%, *P* = .69) between the T1DM + CD and T1DM only groups. In the univariate analysis, CD status was independently associated with fracture (OR 3.88, 95% CI 1.39-10.88, *P* = .01); no associations were observed for duration of T1DM, HbA1c, presence of microvascular complications or vitamin D status with fracture. The relationship between CD and fractures remained significant after adjustment for age, sex, BMI, vitamin D status, hypoglycaemia, microvascular complications and HbA1c (OR 3.50, 95% CI 1.01-12.12; *P* < .05).

3.2 | Hypoglycaemia

Patients with T1DM + CD had a higher prevalence of frequent hypoglycaemia (≥2 episodes per week) compared with T1DM alone (55.1% vs 37.7%, *P* < .001). When hypoglycaemia frequency was stratified by episodes per week, there was a higher proportion of patients with T1DM + CD with 2-4 episodes per week (36.7% vs 22.3%, *P* = .03) and 5-9 episodes per week (14.3% vs 4.3%, *P* = .01) compared with T1DM alone. The proportion of patients with >9 hypoglycaemic episodes per week (4.1% vs 1.2%, *P* = .16), and severe hypoglycaemia (10.2% vs 5.5%, *P* = .20) were similar in both groups (Table 3). In the univariate analysis, duration of T1DM, HbA1c, vitamin D sufficiency and CD status was significantly associated with hypoglycaemia. After adjustment for age, sex, duration of diabetes, HbA1c, BMI and total daily dose of insulin, the relationship between CD status and hypoglycaemia remained significant (OR 3.28, 95% CI 1.61-6.69, *P* = .001). Although age was not associated with hypoglycaemia in the univariate

TABLE 3 Glycaemic control, microvascular complications and hypoglycaemia frequency

	T1 + CD (n = 49)	T1DM only (n = 346)	*P*-value
Any microvascular complication (%)	7 (14.3%)	43 (12.4%)	.65
Retinopathy (%)	7 (14.3%)	42 (12.1%)	.65
Nephropathy (%)	7 (14.3%)	32 (9.2%)	.30
Neuropathy (%)	0 (0%)	9 (2.6%)	.61
Hypoglycaemia (≥2/wk) (%)	27 (55.1%)	96 (27.7%)	**<.001**
2-4 episodes/wk	18 (36.7%)	77 (22.3%)	**.03**
5-9 episodes/wk	7 (14.3%)	4 (4.3%)	**.01**
>9 episodes/wk	2 (4.1%)	4 (1.2%)	.16
Severe hypoglycaemia (%)	5 (10.2%)	19 (5.5%)	.20

Bold values denote significant P-values (P<0.05).

TABLE 4 Univariable analysis and multivariable logistic regression model displaying risk factors for hypoglycaemia

	Univariable analysis				Multivariable logistic regression			
	B	OR	95% CI	*P*-value	*B*	OR	95% CI	*P*-value
Age	−0.13	0.99	0.95-1.03	.51	−0.06	0.94[a]	0.89-0.99	**.03**
Duration of T1DM	0.05	1.05	1.02-1.08	**.002**	0.05	1.05[a]	1.01-1.10	**.01**
HbA1c	−0.26	0.78	0.67-0.89	**<.001**	−0.34	0.71[a]	0.60-0.85	**<.001**
Vitamin D sufficiency	−0.50	0.61	0.38-0.96	**.03**	−0.74	0.48[a]	0.29-0.80	**.005**
Coeliac disease	1.16	3.20	1.74-5.88	**<.001**	1.19	3.28[a]	1.61-6.69	**.001**

B, beta coefficient; OR, odds ratio; CI, confidence interval.
[a]Adjusted for gender, body mass index (per kg/m^2 increase), microvascular complications (absence/presence) and total daily dose of insulin (per international unit increase).
Bold values denote significant P-values (P<0.05).

analysis, this relationship became significant after adjustment for clinically relevant risk factors (OR 0.94, 95% CI 0.89-0.99, *P* = .03) (Table 4).

Higher HbA1c value (OR 0.71, 95% CI 0.60-0.85, *P* < .001) and serum vitamin D level ≥50 nmol/L (OR 0.48, 95% CI 0.29-0.80, *P* = .005) were associated with a reduced risk of hypoglycaemia; the relationships of both covariates with hypoglycaemia were strengthened in the multivariable logistic regression. Conversely, a longer duration of T1DM was observed to increase the risk of hypoglycaemia (OR 1.05, 95% CI 1.01-1.10, *P* = .01) (Table 4). The duration of CD had no effect on hypoglycaemia (*P* = .56).

3.3 | Coeliac disease

Thirty-six of 49 patients (73.5%) in the T1DM + CD group had documented compliance with a GFD. The majority of those who were noncompliant (12 of 13 patients with CD) reported an absence of classical gastrointestinal symptoms due to coeliac disease, and this may partly explain the noncompliance to a GFD. Adherence to a GFD was not associated with hypoglycaemia or fracture risk. Only 11 patients (22.4%) had ongoing gastroenterology follow-up.

4 | DISCUSSION

In this cross-sectional study of young adults with T1DM attending diabetes outpatient clinics at a large tertiary hospital, reported prevalence of fractures was increased in patients with T1DM + CD compared with T1DM alone, with few documented BMD measurements. Reported hypoglycaemia was also increased and independently associated with CD status in T1DM, although glycaemic control and prevalence of microvascular complications were similar between groups. Associations between CD and fracture were significant after adjustment for various risk factors, including age, sex, BMI, vitamin D status, glycaemic control and microvascular complications; however, hypoglycaemia was not independently associated with fracture. Replete vitamin D (≥50 nmol/L) was associated with less hypoglycaemia (OR 0.48, 95% CI 0.29-0.80; *P* = .005), but not with fractures.

Up to 70% of patients with CD have low BMD, with appendicular sites rich in cortical bone being more affected than the axial skeleton.[11] Whilst Australian and British Gastroenterology guidelines have recommended assessment of BMD at baseline,[5,25] an overall consensus has not yet been reached on either the necessity or timing of BMD assessment.[8] Strict adherence to a GFD is the mainstay of treatment in CD and, together with adequate calcium intake and optimal vitamin D, has been shown to restore BMD to normal levels in children, although this may not always be the case in adults.[11] The propensity to fracture is increased in individuals with CD. A recent meta-analysis demonstrated that compared to controls, patients diagnosed with CD had a 69% and 30% increased risk of hip and any fractures, respectively.[12] Studies examining fracture outcomes in adults with T1DM + CD are scarce. A prospective cohort study of over 5000 Swedish patients with T1DM did not demonstrate an increased risk of fracture in those with concomitant CD, compared with T1DM alone.[21] However, there were several limitations of the study, including the large patient age range (4 to 71 years old) incomplete reporting of fractures and lack of information on glycaemic control and adherence to a GFD. In contrast, Weber et al[13] observed a significantly increased fracture risk in females with T1DM + CD compared to those with T1DM alone. (hazard ratio 1.8), in a large population-based cohort study in the United Kingdom. Whilst the absolute risk of fracture in young adults with chronic disease can be difficult to assess, the increased prevalence of fracture in those with T1DM + CD in our study highlights a gap in current literature of bone health and fracture outcomes in this population.

Hypoglycaemia is a complication of insulin therapy in T1DM and leads to considerable morbidity and psychological distress.[23] The phenomenon of increased hypoglycaemia in adults with T1DM + CD was first described in 1978 by Walsh et al[26] who noted "troublesome hypoglycaemia" and unstable glycaemic control in 14 cases, as confirmed in other reports.[27,28] Intestinal mucosal changes associated with CD are thought to interfere with carbohydrate absorption, leading to increased glycaemic variability and hypoglycaemia.[27] In 124 adults with T1DM with severe and recurrent hypoglycaemia who met the criteria for islet cell transplantation, the prevalence of CD was 8%; 7 of 10 patients were newly diagnosed with CD.[29] A nationwide Swedish study reported that the risk of diabetic ketoacidosis and hypoglycaemia was

not increased in T1DM + CD; however, only subjects who required admission to hospital for hypoglycaemia were examined, with the true incidence of hypoglycaemia likely to be underestimated.[30] Our results showing that CD is independently associated with reported hypoglycaemia, is consistent with much of the published literature, showing the need for greater vigilance in T1DM + CD. Here with relatively small numbers reporting noncompliance to a GFD, we did not show a relationship with compliance and hypoglycaemia, yet others have shown that adherence to a strict GFD may benefit patients who suffer from recurrent hypoglycaemia.[27]

Recurrent hypoglycaemic events can lower the hypoglycaemic threshold at which symptoms occur in an individual, due to attenuation of the sympatho-adrenal response.[23] Impaired hypoglycaemic awareness increases the severity of hypoglycaemia and is a significant risk factor for falls and serious injury. Musculoskeletal injuries and vertebral fractures have been reported in patients with T1DM in the setting of hypoglycaemic convulsions.[31] Although we did not observe an independent association between hypoglycaemia and fracture in our study, this can be attributed to reduced statistical power, given the relatively small number of reported fractures.

Vitamin D deficiency is prevalent in individuals with T1DM,[32] and this may be exacerbated by concomitant CD. The proportion of patients with vitamin D insufficiency in our study was 36.6% and 42.2% in the T1DM + CD and T1DM group, respectively. This is comparable to the general Australian population, where up to 50% of Australians are vitamin D deficient, depending on the season.[33] We found that a serum 25-hydroxyvitamin D level of ≥ 50 nmol/L was inversely associated with hypoglycaemia. Only one other study has reported an increased frequency of hypoglycaemia associated with vitamin D deficiency in T1DM, albeit in a paediatric cohort.[34] Although the mechanism by which vitamin D is related to hypoglycaemia is unclear, it has also been linked to adverse cardio-metabolic outcomes in patients with T1DM.[35] Overall, as proposed in other conditions, vitamin D levels may be a surrogate marker of general nutrition and health in this population.

In comparison to a large German-Austrian cohort study by Rohrer et al[10] where CD was found to be an independent risk factor for the development of retinopathy and nephropathy, we did not find an increased risk of microvascular complications in our study. However, the development of retinopathy and nephropathy in patients with T1DM and CD in that study occurred at median diabetes durations of 18.6 and 24.1 years, respectively. In our study, the median duration of diabetes was 14 and 11 years in the T1DM + CD and T1DM groups, respectively, and this may not have allowed sufficient time for microvascular complications to develop.

4.1 | Strengths & limitations

There are several limitations of this study. Firstly, given the observational and retrospective study design, only documented fractures and hypoglycaemic episodes could be captured and data may be incomplete. The correlation of fracture risk to BMD was not possible, as few had bone densitometry. Calcium and PTH levels were not routinely measured. In a cohort at risk of vitamin D deficiency and malabsorption, calcium, PTH levels and other bone turnover markers may provide additional information on bone metabolism. Information on smoking, alcohol consumption and a family history of osteoporosis was not available for all patients. Lastly, the selection of patients attending specialist clinics at a tertiary centre may also reflect a cohort of patient with more severe or complex disease, intrinsically at greater risk of glycaemic instability and bone fragility. Strengths include that to our knowledge, this is the first study examining fracture prevalence and exploring associations with glycaemic control and microvascular complications in young adults comparing T1DM + CD with T1DM alone. We were able to obtain detailed information from individually reviewed medical records with proformas requiring structured recording of hypoglycaemic frequency and of CD status.

5 | CONCLUSION

In a large tertiary outpatient setting, adults with T1DM + CD had increased documented fractures compared to those with T1DM alone, with CD status independently associated with fractures. Despite these risks, few patients had documented bone density measurements. Hypoglycaemia was also increased and independently associated with CD, but not with fractures. Vitamin D ≥ 50 nmol/L was inversely associated with hypoglycaemia, but was not related to fractures. Awareness of bone health in patients with T1DM + CD is important. Whilst this study provides unique data on fracture outcomes and hypoglycaemia in a young adult population, additional longitudinal data including an older cohort with T1DM and CD, with a higher absolute risk of fracture, are warranted. Future prospective cohort studies examining the impact of T1DM and CD on bone density, fracture risk and glycaemic control may help to guide management and fracture risk assessment of these patients.

CONFLICT OF INTERESTS

The authors declare no competing financial interests.

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How to cite this article: Thong EP, Wong P, Dev A, Ebeling PR, Teede HJ, Milat F. Increased prevalence of fracture and hypoglycaemia in young adults with concomitant type 1 diabetes mellitus and coeliac disease. *Clin Endocrinol (Oxf)*. 2017;00:1–7. https://doi.org/10.1111/cen.13488

2.2 SUMMARY & CONCLUSIONS

The risk of fragility fractures is increased in individuals with T1D. Low BMD may be a feature of individuals with T1D, owing to reduced bone turnover, disturbances in calcium and vitamin D metabolism, failure to accrue peak bone mass and concomitant autoimmune disease. Despite the modest reductions in BMD, fracture risk appears to be disproportionately high in this cohort, suggesting that other determinants of bone biomechanical properties and clinical risk factors may contribute to skeletal fragility in T1D. Novel imaging modalities have uncovered deficits in bone microarchitecture and biomechanical properties, which may contribute to skeletal fragility in T1D. These impairments in bone microstructure appear to be associated with poorer glycaemic control and the presence of microvascular complications.

The findings from Chapter 2.1.1 demonstrate that coeliac autoimmunity and T1D confers a greater risk of fracture, compared with T1D alone. Importantly, a quarter of patients with coeliac disease in our study had no gastrointestinal symptoms, and were therefore non-compliant with a gluten-free diet, which is the cornerstone of coeliac disease management. Coeliac disease is also associated with vitamin D deficiency and hypoglycaemia, which can increase the risk for skeletal fragility. The limitations of this study included the cross-sectional design and lack of BMD data, serum calcium and PTH levels. Few individuals with T1D and coeliac disease had ever undergone BMD testing or had routine calcium and PTH levels measured, despite having risk factors for secondary osteoporosis. Hypoglycaemia is an important risk factor for falls, injury and fracture[84,108,109], particularly in individuals with impaired awareness of hypoglycaemia. The case study and literature review in Chapter 2.0.3 illustrates the severity of vertebral fractures that can occur in the setting of a hypoglycaemic seizure, in an individual with T1D with low BMD and impaired bone microarchitecture. This would suggest that fracture risk prevention in T1D should encompass optimization of glycaemic control, with the prevention of hypoglycaemia and falls.

3. CHAPTER 3: CONTEMPORARY RISK OF MENSTRUAL DISORDERS IN TYPE 1 DIABETES

3.0 INTRODUCTION

Historically, the majority of women with T1D exhibited a spectrum of pubertal delay, menstrual and reproductive disorders[54,110,111], in the face of severe metabolic derangement. Ongoing improvements in glycaemic control have ameliorated some of these problems, such as the normalisation of the age at menarche; however, menstrual disorders continue to be prevalent in this cohort, especially in adolescent girls. The prevalence of menstrual dysfunction in women with T1D is less well characterised, although a handful of older studies have reported a higher prevalence of amenorrhea and oligomenorrhea in women with T1D[52,53,110,111], compared with controls. However, the impact of contemporary insulin therapies on menstrual and reproductive disturbance is not clear. Enhanced glycaemic and metabolic control may reinstate normal functioning of the HPO axis and reduce glucose toxicity to ovarian tissues, thus restoring ovulatory cycles.

On the other hand, intensification of insulin therapy is associated with weight gain and obesity[112], which confers significant cardio-metabolic risk. Furthermore, obesity itself is associated with disorders of reproduction, including menstrual cycle alterations, infertility and PCOS[113]. Overall, the uptake of intensive insulin therapy and improved glycaemic control appear to be implicated in the emergence of PCOS in T1D[60,61], and although not previously framed as such, it is conceivable that T1D could induce 'secondary' PCOS[45].

3.1 OBESITY, MENSTRUAL IRREGULARITY AND POLYCYSTIC OVARY SYNDROME IN YOUNG WOMEN WITH TYPE 1 DIABETES: A POPULATION-BASED STUDY

(Submitted to *The Lancet Diabetes & Endocrinology*, March 2020, under review)

Obesity, menstrual irregularity and polycystic ovary syndrome in young women with type 1 diabetes: a population-based study

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Running title: Obesity and menstrual disorders in T1D

Keywords: Type 1 diabetes; obesity, menstrual disorders, PCOS

Word count: Abstract (307 words), Main body (3797 words)

Number of tables/figures: 5

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Conflicts of Interest: The authors declare no conflicts of interest relevant to this article.

ABSTRACT

Background: Poor glycaemic control in type 1 diabetes (T1D) disrupts the hypothalamic-pituitary ovarian axis, leading to hypothalamic amenorrhea and infertility. These reproductive disturbances can be ameliorated with improved glycaemic control at the cost of increased exogenous insulin and rising obesity. Reproductive implications of adiposity and increased insulin are now emerging, including polycystic ovary syndrome (PCOS).

Aims: To evaluate changes in body mass index (BMI), and the relationship between obesity, menstrual irregularity and polycystic ovary syndrome (PCOS), in young women with T1D.

Methods: Longitudinal observational study using data from the Australian Longitudinal Study in Women's Health (ALSWH) of the cohort born in 1989–95, from 2013 to 2015. Prevalent and incident menstrual irregularity and PCOS were evaluated, along with BMI changes over two years. Random effects logistic regression analysis was used to evaluate risks of menstrual irregularity and PCOS.

Results: Overall, 15926 women were included at baseline (T1D, n=115; controls, n=15811). Mean age was similar between groups (20·7 vs. 20·5 years, p=0.38), however median body mass index (BMI) was increased in women with T1D (25·5 vs. 22·9 kg/m^2, p<0·001). Over half of women with T1D were overweight or obese (54·4% vs. 32·9%, p<0·001). Median BMI increased by 1·11 and 0·49 kg/m^2, in the T1D and control groups, respectively. At baseline, the prevalence of PCOS was higher in the T1D group (16·5 vs. 5·5%, p<0·001). T1D was independently associated with an increased risk of menstrual irregularity (RR 1·22, 95%CI 1·02–1·46) and PCOS (RR 2·41, 95%CI 1·70–3·42). Independent of T1D status, obesity conferred a 4-fold increased risk of PCOS, compared to those with a BMI in the normal range (RR 3·93, 95%CI 3·51–4·42).

Conclusions: Obesity is prevalent amongst women with T1D, and may be a key contributor to the higher risk of menstrual irregularity and PCOS in this cohort, representing an important opportunity for prevention and intervention.

Research in context

Evidence before this study

Menstrual and reproductive disorders are common in women with type 1 diabetes, affecting up to 40% of women in this group. Perturbations of the hypothalamic-pituitary-ovarian axis, arising from uncontrolled hyperglycaemia and metabolic disruption, have been identified in these women, who frequently exhibit amenorrhea and oligomenorrhea. Improvements in diabetes management, such as the intensification of insulin therapy in the early 1990's, have resulted in better metabolic control; however, menstrual disorders remain highly prevalent in contemporary cohorts of young women with type 1 diabetes. In particular, there appears to be a rise in the prevalence of polycystic ovary syndrome, a condition typically associated with type 2 diabetes, obesity and metabolic dysfunction. A systematic review and meta-analysis by Escobar-Morreale and colleagues in 2016 reported a pooled prevalence of polycystic ovary syndrome in 24% of adolescent and adult women with type 1 diabetes, in nine included studies. Importantly, the increasing rates of obesity in type 1 diabetes in recent years may influence the association with polycystic ovary syndrome. We replicated this systematic search on PubMed, Google Scholar and the Springer Online Archives Collection for articles published up to March 1, 2020, and identified four new studies confirming the increased prevalence of irregular menses and polycystic ovary syndrome amongst reproductive-aged women with type 1 diabetes. Two of these studies examined the association of body mass index on polycystic ovary syndrome, with one study demonstrating higher body mass index in women with type 1 diabetes and polycystic ovary syndrome, and the other reporting no

association of body mass index with anti-Müllerian hormone measurements, a biomarker correlated with polycystic ovary syndrome.

Added value of this study

We performed a large-scale, longitudinal, population-based cohort study to evaluate changes in body mass index and the risk of menstrual irregularity and polycystic ovary syndrome in type 1 diabetes. Here, we show a 50% prevalence of overweight and obesity in young women with type 1 diabetes, which was significantly higher than controls. These women had a higher body mass index at baseline, which appeared to increase with time. Type 1 diabetes and obesity independently conferred increased risks of menstrual irregularity and polycystic ovary syndrome. Weight gain and rising obesity rates in contemporary type 1 diabetes cohorts has adverse reproductive implications, and represents an opportunity for monitoring and prevention of weight gain.

Implications of all the available evidence

While contemporary type 1 diabetes management has afforded better metabolic control, the adverse effect of weight with intensive insulin regimens may negate this benefit. Increases in body mass index appear to drive the increase risk of menstrual irregularities and polycystic ovary syndrome, which may have important ramifications on fertility and long-term cardio-metabolic sequelae in young women with type 1 diabetes. Greater awareness of reproductive disorders in type 1 diabetes is needed, with the assessment of reproductive health an important consideration in young women with type 1 diabetes, particularly in those who are overweight or obese.

Introduction

Type 1 diabetes mellitus (T1D) is a chronic autoimmune condition with a peak incidence in childhood and adolescence worldwide[1]. In Australia, 61% of all new diagnoses of T1D in 2017 occurred in individuals under the age of 25, where the age of diagnosis peaked amongst those aged between 10 to 14 years[2]. The onset of T1D at an early stage in the lifespan, particularly at a prepubertal age, has ramifications on the gonadotrophic axis and ovarian function, with as many as 40% of females with T1D exhibiting menstrual and reproductive disorders[3]. Reproductive disturbance is therefore common in young women with T1D, encompassing a spectrum of menarchal delay, oligomenorrhea, polycystic ovary syndrome (PCOS) and early menopause.

Perturbations of the hypothalamic-pituitary-ovarian axis have been described in individuals with T1D[3-5]. While the mechanisms for reproductive disturbance in T1D are not well understood, multiple factors are likely to contribute to the overall pathogenesis. Insulin plays an important role in the regulation of ovarian function and the gonadotrophic axis. A state of insulin deficiency, as in the case of T1D, has been shown to decrease pituitary gonadotrophin-releasing hormone (GnRH) stimuli, leading to downstream effects of reduced luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion, and consequently, reduced ovarian steroid production[4,6]. Hyperglycaemia exerts gluco-toxic effects on the ovaries directly, or indirectly via advanced glycation end-products and their receptors[7]. Increased apoptosis of follicular and granulosa cells, dysfunction of oocyte maturation and ovarian steroidogenesis have been observed with insulin deficiency and hyperglycaemia in animal models of T1D[8]. Poor metabolic control of T1D is associated with a catabolic state, characterized by low bodyweight and a reduction of leptin, an important hormone regulator of energy balance, which can further suppress hypothalamic

gonadotrophin secretion[3,4]. Prior to the advent of insulin therapy, the majority of patients with T1D failed to thrive and exhibited profound hypogonadism, with severe pubertal delay, amenorrhea and infertility[3].

The discovery and introduction of insulin therapy in 1922 led to improved metabolic control, which ameliorated amenorrhea and subfertility in women with T1D[9]. However, up until the late 1980s, menstrual disturbance and menarchal delay were highly prevalent, predominantly in women diagnosed with T1D before puberty[10,11]. In 1993, the landmark Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study demonstrated the benefits of intensive insulin treatment, comprising three or more daily injections of insulin or pump therapy, on the onset and progression of long-term diabetic microvascular sequelae[12]. With enhanced metabolic control, menarchal delay is now uncommon in girls with T1D[13,14], although oligomenorrhea continues to persist in 25 – 35%[15-17] of adolescent girls and around 20%[18,19] of adult women with T1D. Menstrual irregularities in this cohort have been associated with poorer glycaemic control and weight gain[15,20].

Importantly, there has been an emergence of polycystic ovary syndrome (PCOS) in T1D over the last two decades. In 1994, Adcock and colleagues found that over two-thirds of post-menarchal girls with T1D who had irregular menses, exhibited polycystic ovarian morphology[20]. Subsequently, in 2000 Escobar-Morreale and colleagues reported a high prevalence of hyperandrogenism and PCOS in women with T1D[21]. Several other studies[22,23] have since confirmed these findings, where the pooled prevalence in a recent meta-analysis was reported to be 24% in women with T1D[21], compared to 8-13% in the general population[24,25]. The emergence of PCOS appears to mirror the increase in obesity rates over

the last few decades, in this cohort. Obesity and PCOS appear to have a bidirectional interaction, each significantly exacerbating the other condition[26-28]. Few studies have explored the contribution of weight gain, overweight and obesity to menstrual disturbance and PCOS in this cohort. Therefore, we sought to evaluate the relationships between BMI on menstrual irregularity and PCOS, respectively, in young women with T1D.

Methods

Study population

The Australian Longitudinal Study in Women's' Health (ALSWH) is a national initiative funded by the Australian Government Department of Health, to evaluate sociodemographic, psychological and behavioral factors and their impact on women's health and well-being across different life stages. Further information on the ALSWH can be found elsewhere[29]. At its inception in 1996, three cohorts of Australian women, born in 1921–26, 1946–51 and 1973 – 78, were randomly selected from the national Medicare database, who participated in the study via mailed questionnaires. In 2012 to 2013, a fourth cohort of women born in 1989–95, was established to provide contemporary health information about women in early adulthood. For the present study, we utilized data from this group of women, aged 18 to 23 years[30-32]. Recruitment for this cohort took place predominantly via the internet and social networking websites. Follow-up online questionnaires were administered every year thereafter, and the study period comprised 3 questionnaires at baseline (Survey 1), Year 1 (Survey 2) and Year 2 (Survey 3), from 2013 to 2015.

Self-reported outcomes

Women were classified as having T1D if they reported ever having 'been diagnosed or treated for type 1 (insulin-dependent) diabetes mellitus'. Those who reported a diagnosis of type 2 diabetes mellitus (T2D) or pre-diabetes (impaired glucose tolerance or impaired fasting glycaemia), were excluded from the study, as were women with other self-reported major chronic physical illness such as malignancy, cystic fibrosis, chronic neurological, renal, liver disease, previous hysterectomy and/or bilateral oophorectomy. Women with specific conditions associated with hypogonadism, such as eating disorders, including anorexia nervosa, bulimia nervosa and eating disorders not otherwise specified, Turner's syndrome and Fragile X syndrome, were also excluded.

Demographic information was collected at baseline, including age, place of residence (city, regional or rural areas), alcohol use, cigarette smoking and highest level of education. Women were asked if they had prior diagnoses of medical and psychiatric conditions, such as hypertension, thyroid disease, coeliac disease, anxiety and depression. BMI was derived from self-reported height and weight, which was collected at every survey. Four BMI categories were established: underweight (<18·5 kg/m^2); normal weight (18·5 to <25 kg/m^2); overweight (25 to <30 kg/m^2); and obese (⩾30 kg/m^2), according to the World Health Organization classifications. Information on physical activity was also collected at every survey, measured by calculating metabolic equivalent (MET) minutes per week, derived from the amount of time spent doing walking, moderate and vigorous activities. Physical activity was categorized as 'sedentary' (MET minutes < 40/week), 'low' (MET minutes 50 – <600/week), 'moderate' (MET minutes 600–1200/week) and 'high' (MET minutes greater than 1200/week).

Reproductive outcomes

Information on age of menarche, contraceptive use and pregnancy history were collected from Survey 1 at baseline. The primary outcomes of interest were self-reported menstrual irregularity and PCOS diagnosis ('have you ever been diagnosed with or treated for polycystic ovary syndrome?'), which were collected at baseline, and at every survey thereafter. For each of the menstrual disorders, namely irregular periods, heavy periods and severe period pain, women were asked if they had experienced this in the last 12 months, at every survey. Categorical responses of 'never' or 'rarely', and 'sometimes' and 'often' were recoded into binary outcomes as 'no' and 'yes', respectively. Women who reported menstrual irregularity and PCOS from Survey 2 (Year 1) onwards were considered as incident cases, if they had responded 'no' to having irregular periods or PCOS at baseline.

Statistical analyses

All statistical analyses were performed using Stata/IC 15.1 for Windows. Continuous variables were presented as means (± standard deviation) or medians (with interquartile range) and compared using the student's t-test or Wilcoxon rank-sum test, for parametric and non-parametric data, respectively. Categorical data were presented as percentages and compared using the chi-squared test. Comparisons were performed to evaluate changes in BMI at baseline and Year 1, Year 1 and Year 2, baseline and Year 2 for both the T1D and control groups, using the paired samples Wilcoxon signed-rank test. Log-binomial regression models were used to estimate risk ratios for incident menstrual irregularity and PCOS. Univariable regression analysis was first performed, with variables retained at $p < 0{\cdot}1$. The final multivariable log-binomial regression model incorporated adjustment for T1D status, age, BMI category, age at menarche and oral contraceptive use. Statistical significance was defined as $p<0{\cdot}05$.

Results

Baseline demographics and BMI

Overall, 17069 Australian women aged 18–23 years responded to the first web-based survey in 2012–13. At baseline, 15926 women were included (T1D, n=115; controls, n=15811). Mean age was similar in both T1D and control groups (20·7±1.7 vs. 20·5±1.7, p=0·38). Median weight (70kg, IQR 63 – 78 vs. 64kg, IQR 56 – 74, p<0.001) and BMI (25·5, IQR 23·1–28·2 vs. 22·9, IQR 20·5–26·4 kg/m^2, p<0·001) were higher in the T1D group. Despite no differences in physical activity levels between groups, more than half of women in the T1D group had a BMI in the overweight or obese category, compared to a third in the control group (54·4% vs. 32·9%, p<0·001). There were no differences in smoking or alcohol use between groups. Over two-thirds of women in both groups were living in major cities, and a quarter had completed tertiary education. Medical and psychiatric comorbidities, such as hypertension (8·7% vs. 1·7%, p<0·001), coeliac disease (2·6% vs. 0·5%, p<0·001), thyroid disease (3.5% vs. 0.5%, p<0.001) and depression (47·8% vs. 33·4%, p<0·001) were significantly more prevalent in women with T1D (Table 1).

Reproductive characteristics, contraception use and PCOS status

Mean age at menarche was similar at 12·8 years in both groups. Menstrual disorders, namely irregular periods, heavy periods and dysmenorrhea were highly prevalent in both groups, with 47·8% of women with T1D and 40·3% of controls reporting menstrual irregularity at baseline. PCOS was significantly more prevalent in women with T1D (16·5 vs. 5·5%, p<0·001). More women with T1D reported prior pregnancy (21·7 vs. 13·2%, p=0·007) and miscarriage (12·2 vs. 4·3%, p<0·001), compared to controls. The uptake of contraception was significantly lower in women with T1D (77·9% vs. 87·0%, p=0·008). Over half of women in both groups

used oral contraceptive pills, which was not different between groups. A higher proportion of women in the control group used barrier contraception (31·6 vs. 42·6%, p=0·03); however, more women with T1D reported intra-uterine device use (6·3 vs. 2·0%, p=0·002) [Table 2].

BMI changes, menstrual irregularity and PCOS incidence

At the end of Year 2, 61 women with T1D and 8332 controls remained in the study. BMI differences at baseline were significant between the 115 women with T1D and 15811 controls (25·5, IQR 23·1–28·2 vs. 22·9, IQR 20·5–26·4 kg/m^2, p<0.001). Changes in BMI over the study period were evaluated only in women who had BMI measurements at all t``hree time points. BMI remained stable in both the T1D and control groups between baseline and Year 1, but increased significantly between Year 1 and Year 2 in both groups. Notably, BMI increased by 0·39 kg/m^2 in the T1D group (p=0·001) and 0·34 kg/m^2 in the control group (p<0·001), during this year, equating to median weight increases of 1·5 and 1 kg, respectively. Overall, by Year 2, the net change in median BMI was 1·11 kg/m^2 in the 61 women with T1D (p=0·04) and 0·49 kg/m^2 in the 8332 controls (p<0·001), correlating with median weight increases of 2 and 3 kg over two years, respectively (Table 3).

Over two years, 3220 controls and 31 women with T1D reported new menstrual irregularity. On univariable analysis, the relative risk of menstrual irregularity was 1.38 in women with T1D (95% CI 1·05–1·81, p=0·019). After adjustment, T1D and obesity were independently associated with a 22- and 37-percent increased risk of menstrual irregularity, respectively. Additionally, underweight and overweight were both associated with a 9% increased risk of menstrual irregularity, while oral contraceptive pill use and younger age appeared to be protective factors (Table 4).

Overall, 910 incident cases of PCOS were reported (T1D, n=11; controls, n=899) over two years of follow-up. T1D was associated with a 2·5-fold increased risk of PCOS on the unadjusted analysis (95%CI 1·73–3·73, p<0·001). Although menstrual irregularity was highly correlated with PCOS in univariable analysis, this variable was omitted from the multivariable logistic regression model due to collinearity, as menstrual irregularity is a key diagnostic criterion for PCOS. In the final analysis, T1D conferred a 2·4-fold increased risk of PCOS, while obesity was independently associated with a 4-fold increased risk of PCOS (Table 5).

Discussion

Overweight and obesity, along with menstrual disorders, were highly prevalent amongst this unselected population-based cohort of young women with T1D. At baseline, PCOS was more prevalent and median BMI higher in women with T1D, compared to controls. Overall, BMI in both groups increased significantly between Year 1 and Year 2 . T1D, overweight and obesity independently conferred increased risks for both menstrual irregularity and PCOS. To our knowledge, this is the first study to assess BMI changes, menstrual irregularity and PCOS and the relationships between these, in a contemporary T1D cohort.

Over half of women with T1D in our study were overweight or obese. These findings are consistent with another Australian study of 501 adults with T1D, where the reported prevalence of overweight and obesity was 38% and 15%, respectively[33]. Previously rare in T1D, the increasing prevalence of obesity in this cohort is only partially explained by the global obesity epidemic, and is cause for concern. Temporal trends have shown an increase in T1D obesity rates from 3% in the 1980s to 23% in the mid-2000s, which surpassed the increases seen in the general population[34]. In our study, increases in median BMI were seen

in both T1D and control groups between Year 1 and 2. This was not explained by changes in physical activity levels. The rise in obesity in T1D appears to coincide with the uptake of intensive insulin therapy over the last few decades, with the prevalence of obesity in DCCT/EDIC increasing from 1% at baseline in 1983-1989, to 31% after 12 years, in the group originally randomized to intensive insulin therapy[12]. While DCCT/EDIC demonstrates that intensive insulin therapy has clear benefits on glycaemic control and preventing diabetes-related complications, an increase in cardiovascular events was reported after 14 years, in those who were at the highest quartile of weight gain, where the BMI increase was in excess of 6kg/m^2 [35]. The increased prevalence of overweight and obesity in women with T1D here, is concerning, and more studies are needed to evaluate BMI trajectories over a longer period. As obesity exacerbates insulin resistance, cardio-metabolic and as shown here, reproductive complications in T1D, awareness, monitoring and weight gain prevention are important in the context of intensive insulin therapy and rising weight gain[36].

Earlier studies have reported an increased risk of menstrual irregularity in adolescents and women with T1D that was attributable to hypogonadotrophic hypogonadism, underpinned by metabolic disruption. While menarchal delay is now uncommon with improved glycaemic control under contemporary treatment, menstrual disorders continue to be prevalent in young women with T1D. Oligomenorrhea and increased cycle length are the most common menstrual cycle abnormalities seen in contemporary T1D cohorts, affecting 30% of adolescents with T1D[16,37] and around 20 to 40% of women with T1D[19,38]. Our finding of nearly 50% of women with T1D reporting menstrual irregularity is slightly higher than figures reported in previous studies, and is not explained by differences in uptake of hormonal contraception. While T1D status conferred a 1.5-fold increased risk of menstrual irregularity, overweight and obesity were also independent contributors to menstrual

irregularity. The higher rate of menstrual irregularity in women with T1D may be partially attributable to the high prevalence of overweight and obesity in women with T1D in our study, particularly those with PCOS. Both uncontrolled hyperglycaemia and exogenous insulin exposure can adversely affect ovarian function, giving rise to menstrual irregularity. Further research is therefore needed to delineate the cause of ovulatory and menstrual dysfunction in this cohort, given the long-term implications of hypogonadism and PCOS on bone and cardiovascular health, respectively.

PCOS is the most common endocrinopathy in women of reproductive age, and is traditionally associated with insulin resistance, obesity and type 2 diabetes. Intensive insulin therapy has been implicated in the rise of PCOS in young women with T1D, via postulated mechanisms of exogenous hyperinsulinaemia and weight gain. Subcutaneous administration of insulin bypasses the hepatic portal circulation, leading to increased insulin levels in the systemic circulation, which exerts a stimulatory effect on ovarian follicles to increase androgen production[39,40]. Weight gain, a potential side effect of intensive insulin therapy[41], can further contribute to menstrual irregularity and PCOS in this cohort, especially during the pubertal transition, when insulin resistance is increased[42]. Our reported PCOS prevalence of 16.5% in women with T1D is slightly lower than the pooled prevalence of 24% in the meta-analysis by Escobar-Morreale and colleagues, and may be due to discrepancies in diagnostic criteria used. Importantly, here we have also shown that the risk of PCOS was greater than 2-fold in women with T1D, which was independent of BMI category. Notably, obesity conferred a 4-fold increased risk of PCOS, which is consistent with established associations outside T1D, between obesity and PCOS, where 30 to 70% of women with PCOS are obese[28]. Obesity not only contributes to hyperandrogenaemia and exacerbates the cardio-metabolic features of PCOS[43], but also increases cardiovascular risk[44] and retinopathy[33] in individuals with T1D.

Presently, little is known regarding the long term cardio-metabolic sequelae of PCOS in obese women with T1D, although there is strong evidence to support the efficacy of weight loss in ameliorating the clinical and biochemical features of PCOS[45,46].

We identified several limitations of this study, namely, the use of self-reported outcomes for T1D and PCOS. The diagnostic criteria used to diagnose PCOS was not known, and clinical or biochemical information pertaining to duration of T1D, insulin regimen and doses, glycaemic control and androgen profile was not available. We were not able to establish the cause of menstrual irregularity. BMI was calculated using self-reported weight and height, which could be prone to recall bias. However, substantial agreement between self-reported and measured height and weight[47], and the validity of self-reported PCOS diagnosis[48], have been demonstrated in other cohorts of the ALSWH. Finally, the number of women with T1D relative to controls are small, though this approximates the 0·5% prevalence of T1D in Australia. There are a number of strengths to this study, including a large sample size, longitudinal design with repeated measures, which allowed changes in BMI and associations between menstrual disorders to be examined. In addition, we were able to collect comprehensive lifestyle data in an unselected group of community-dwelling women with T1D.

Conclusions

Here in a large-scale longitudinal community-based cohort, we have shown that women with T1D have higher BMI than controls, which appeared to increase over time. Menstrual disturbances were more common in women with T1D, as was PCOS, which appears to be strongly driven by the rise in BMI. The rising BMI of contemporary T1D cohorts has adverse reproductive implications, and represents an opportunity for monitoring and prevention of

weight gain. Increased awareness of reproductive disorders in T1D is needed amongst clinicians, with consideration given to assessing reproductive health in young women with T1D, particularly in individuals with increasing or high BMI. Further longitudinal studies evaluating the relationship between insulin dosages, glycaemic control, weight trajectories and menstrual disorders, are needed to inform management strategies.

Author Contributions

EPT designed the study, performed the data analyses and wrote the manuscript. FM, AJ, GM and HT assisted in study conception and manuscript revisions.

Acknowledgements

The authors would like to acknowledge that the research from this study was generated from the Australian Longitudinal Study on Women's Health, undertaken by the University of Newcastle and The University of Queensland, funded by the Australian Government Department of Health. We are grateful to the women who participated in the survey.

Funding

This study was not funded. EPT is supported by a Research Training Program Scholarship. FM is supported by an Endocrine Society of Australia Ken Wynne Post-doctoral Research Award. AJ is supported by a National Health and Medical Research Council (NHMRC) Early Career Fellowship. GDM is supported by an NHMRC Principal Research Fellowship. HT is an NHMRC/MRFF Practitioner Fellow.

Duality of Interest

The authors declare no conflicts of interest relevant to this article.

Tables and Figures

	Controls (n=15811)	T1D (n=115)	P-value
Mean age (years)	20·5±1·7	20·7±1·7	0·38
Median weight (kg)	64 (56, 74)	70 (63, 78)	**<0·001**
Median height (cm)	166 (162, 171)	167 (162, 173)	0·50
Median BMI (kg/m^2)	22·9 (20·5, 26·4)	25·5 (23·1, 28·2)	**<0·001**
BMI category (%)			**<0·001**
Underweight (<18.5kg/m^2)	7·4%	1·8%	
Normal (18.5 to <25kg/m^2)	59·8%	43·9%	
Overweight (25 to <30kg/m^2)	19·4%	40·4%	
Obese (≥30kg/m^2)	13·5%	14·0%	
Physical activity category			0·71
Inactive (%)	6·2%	8·7%	
Low (%)	24·6%	25·2%	
Moderate (%)	21·4%	20·0%	
High (%)	47·8%	46·1%	
Current smoking (%)	18·9%	22·6%	0·31
Current alcohol use (%)	96·9%	96·5%	0·84
Mean number of standard drinks/week	1·9±10	1·9±1·1	0·82
Living in major city (%)	74·2%	69·3%	0·23
Tertiary education (%)	29·5%	24·4%	0·23
Hypertension (%)	1·3%	8·7%	**<0·001**
Iron deficiency (%)	31·2%	37·5%	0·25
Thrombosis (%)	0·2%	3·5%	**<0·001**
Coeliac disease (%)	0·5%	2·6%	**<0·001**
Thyroid disease (%)	0·5%	3·5%	**<0·001**
Anxiety (%)	30·4%	27·0%	0·41
Depression (%)	33·4%	47·8%	**<0·001**

Table 1. Baseline demographics

	Controls (n=15811)	T1D (n=115)	P-value
Mean age at menarche (years)	12·8±1·6	12·8±2·1	0·79
Irregular periods (%)	40·3%	47·8%	0·10
Heavy periods (%)	39·6%	48·7%	0·14
Severe period pain (%)	49·0%	54·8%	0·46
PCOS (%)	5·5%	16·5%	**<0·001**
Endometriosis (%)	3·3%	5·2%	0.25
Contraceptive use (yes)	87·0%	77·9%	**0·008**
Ever pregnant (%)	13·2%	21·7%	**0·007**
Ever had a miscarriage (%)	4·3%	12·2%	**<0·001**
Oral contraceptive (%)	54·6%	50·5%	0.43
Barrier contraceptive (%)	42·6%	31·6%	**0·03**
Implanon (%)	10·3%	13·7%	0.28
IUD (%)	2·0%	6·3%	**0·002**
Other contraceptive (%)	3·3%	3·2%	0.96

Table 2. Reproductive characteristics at baseline

	Paired samples comparison of change in median weight, kg (IQR)^		Paired samples comparison of change in median BMI, kg/m^2 (IQR)^	
	Controls (n=8332)	**T1D (n=61)**	**Controls (n=8332)**	**T1D (n=61)**
Baseline to Year 1	0 (-11, 13)	2.5 (-6, 11.5)	0.31 (-3.70, 4.32)	0.41 (-1.78, 3.01)
Year 1 to 2	1 (-1, 3)**	1.5 (-0.5, 3.5)**	0.34 (-0.37, 1.26)**	0.39 (0, 1.26)**
Baseline to Year 2	2 (-10, 14)**	3 (-4, 13)*	0.49 (-3.78, 4.80)**	1.11 (-1.62, 3.50)*

Table 3. Change in median weight and BMI

Asterisks denote significant difference within the same group; ** denotes p<0.01, * denotes p<0.05

	RR	95% CI	p-value
Age^	0·91	0·90 – 0·92	**<0·001**
T1D^	1·22	1·02 – 1·46	**0·033**
BMI category^*			
Underweight	1·09	1·02 – 1·17	**0·018**
Overweight	1·09	1·04 – 1·14	**0·001**
Obese	1·37	1·30 – 1·43	**<0·001**
OCP use^	0·76	0·73 – 0·79	**<0·001**

Table 4. Log-binomial regression analysis for irregular periods

^Model adjusted for T1D, BMI category, age, OCP use and menarche

*BMI category of 18·5 – 24·9 kg/m^2 used as reference category

	RR	95% CI	p-value
Age^	1·04	1·02– 1·07	**0·002**
T1D^	2·41	1·70 – 3·42	**<0·001**
BMI category^*			
Underweight	1·12	0·87 – 1·44	0·37
Overweight	1·77	1·55 – 2·02	**<0·001**
Obese	3·93	3·51 – 4·42	**<0·001**

Table 5. Log-binomial regression analysis for PCOS

^Model adjusted for T1D, BMI category, age, OCP use and menarche

*BMI category of 18·5 – 24·9 kg/m^2 used as reference category

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3.2 SUMMARY & CONCLUSIONS

The ASLWH is an Australia-wide longitudinal observational study designed to study factors related to women's physical, mental and psychological well-being. Data from the cohort of women born in 1989 – 1995 was extracted for this prospective study of reproductive-aged young women, to evaluate changes between BMI and the incidences of menstrual irregularity and PCOS, over two years. There was a significantly higher prevalence of overweight and obesity in women with T1D at baseline, where over 50% had a BMI ≥25 kg/m^2 compared with 32.9% in the control group ($p < 0.001$). Median BMI was in the overweight range in women with T1D at baseline, and increased by 1.11 kg/m^2 over two years. PCOS prevalence at baseline was significantly higher in the T1D group (16.5 vs. 5.5%, $p<0.001$). T1D conferred 1.2- and 2.4-fold increased risks of menstrual irregularity and PCOS in young women, respectively, which may be exacerbated by overweight and obesity.

Overweight and obesity are highly prevalent amongst young women with T1D. BMI in this study cohort was increased in excess of 2.6 kg/m^2, compared with non-diabetic controls. A similar prevalence of overweight and obesity was reported in an earlier Australian study of adults with T1D[114]. Overall, the rate of obesity is rising in T1D, with temporal trends of BMI surpassing that of the increases seen in non-diabetic individuals[115]. The reasons for this phenomenon is not completely clear, although the widespread uptake of intensive insulin therapy has been implicated in weight gain, where individuals randomised to the intensive treatment arm of DCCT gained significantly more weight compared with those randomised to conventional therapy[112]. Furthermore, the prevalence of obesity increased from 1% in the intensive group at baseline, to 31%, after 12 years[116]. Increased BMI is an independent predictor of menstrual irregularity[117] and PCOS[118], and may contribute to increased cardiovascular morbidity in T1D. Although intensive insulin therapy enhances glycaemic control, the risk of weight gain and hypoglycaemia is also increased. Weight gain associated with contemporary management of T1D is likely to exacerbate pre-existing menstrual

dysfunction, or give rise to new menstrual disorders, such as in the case of PCOS. This study was limited by the lack of clinical and biochemical information pertaining to T1D control, insulin doses and androgen profile. In addition, the cause of menstrual irregularity, whether due to HH or PCOS, was not established. Further studies monitoring weight trajectories, insulin doses and PCOS incidence over a longer period, are needed to confirm the impact of intensive insulin therapy on weight gain and menstrual disorders. This study contributes novel longitudinal data showing significantly higher prevalence of reproductive disorders in women with T1D, related to disproportionately rising BMI, and highlights the clinical need for awareness, prevention, screening and management of reproductive disorders in type 1 diabetes, to avoid negating the benefits of tight glycaemic control.

4. CHAPTER 4: INTERACTIONS BETWEEN BONE AND REPRODUCTIVE HEALTH IN TYPE 1 DIABETES

4.0 INTRODUCTION

The relationship between reproductive and bone health in T1D has been previously discussed. Earlier observational cohort studies have reported later menarche and earlier menopause in women with T1D[72,119], representing a potential decrease in reproductive years, and consequent reduction in lifetime oestrogen exposure. Although fracture risk is increased throughout the lifespan in T1D, there appears to be an exponential rise in fracture incidence after the age of 40 in women that continues to rise with age, which is in excess of that of the general female population[33]. These findings are likely attributable to menopause, where loss of bone mass follows the depletion of ovarian reserves and consequent oestrogen deficiency. In a large multi-ethnic study of women undergoing menopause, Greendale and colleagues demonstrated a 10% loss of BMD at the lumbar spine and femoral neck over 10 years. The majority of bone loss occurred in the year preceding and two years after the final menstrual period, termed the “transmenopause”[120]. In women with T1D undergoing the menopause transition, fracture risk could be substantially elevated during this period. To date, only one study has evaluated changes in BMD across the menopause transition in women with diabetes. In this study, Khalil and colleagues[121] reported a greater decline in bone mass at the hip and a higher risk of fractures in women with diabetes, compared with controls. Notably, the age of menopause in women with diabetes was significantly earlier than that of controls. Therefore, reproductive factors, such as age of menarche and menopause, may contribute to skeletal fragility in women with T1D.

4.1 ASSOCIATIONS BETWEEN REPRODUCTIVE AND BONE HEALTH IN WOMEN WITH DIABETES: A 15-YEAR LONGITUDINAL STUDY

(Submitted to the *Journal of Clinical Endocrinology & Metabolism*, Mar 2020, under review)

Associations between reproductive factors and bone health in women with diabetes: a 15-year longitudinal study

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Running title: Association between reproductive and bone health in T1D

Keywords: Type 1 diabetes; bone health; menopause

Word count: Abstract (249 words), Main body (3826 words)

Number of tables/figures: 5

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Conflicts of Interest: The authors declare no conflicts of interest relevant to this article.

ABSTRACT

Context: Skeletal fragility is associated with diabetes mellitus, while limited estrogen exposure during the reproductive years also predisposes to lower bone mass and higher fracture risk.

Objective: To explore osteoporosis diagnosis, fall and fracture rates in women with type 1 (T1D) and type 2 (T2D) diabetes mellitus, and associations with reproductive lifespan and age at menopause.

Design: Prospective observational data drawn from the Australian Longitudinal Study in Women's Health (ALSWH) from 1996 to 2010.

Participants: Women were randomly selected from the national health insurance database. Standardized data collection occurred at six survey time points.

Setting: General community.

Main outcome measures: Self-reported osteoporosis diagnosis, incident fracture and reproductive lifespan.

Results: Overall, 11,313 women were included at baseline (T1D, n=107; T2D, n=333; controls, n=10,873). Over 15 years of follow-up, 885 new cases of osteoporosis and 1,099 incident fractures were reported. T1D was independently associated with 2.7-fold increased odds of osteoporosis (OR 2.66, 95% CI 1.27 – 5.58). T2D (OR 2.49, 95% CI 1.64 – 3.78) and falls (OR 2.17, 95% CI 1.80 – 2.61) were independently associated with fracture. More women with T1D and T2D reported falls (58.8% and. 52.4% vs. 45.4%, p=0.004) compared with controls. Women with T1D had a shorter reproductive lifespan (34.8±3.7 vs. 37.1±5.0 and 37.4±4.0 years, p<0.001).

Conclusions: Women with T1D and T2D have increased self-reported osteoporosis, falls and fractures. A shortened reproductive lifespan, reduced estrogen exposure and increased falls are likely to contribute to fracture risk in T1D, and falls are likely contributors in T2D, presenting opportunities for prevention.

Introduction

Diabetes mellitus is rapidly becoming a global pandemic. In 2000, the global prevalence of diabetes was estimated at 151 million adults. Today, this number has tripled, with 463 million adults and 1.1 million children living with diabetes. By 2030, the global prevalence of diabetes is projected to surpass 578 million[122].

The exponential rise of diabetes represents a severe threat to global health. Type 1 diabetes (T1D) and type 2 diabetes (T2D) increase the risk of health complications, including cardiovascular, kidney and eye disease, in turn posing significant economic challenges to countries, health systems and individuals[122]. In addition to well-established conventional macro- and microvascular sequelae, there is growing recognition of skeletal fragility as a complication of both T1D and T2D. Several meta-analyses[37,38,123] and large cohort studies[33,34,124] have confirmed a propensity for fracture in individuals with T1D and T2D, compared with non-diabetic controls. In particular, hip fracture risk is increased by up to seven[37]- and two-fold[38] in T1D and T2D, respectively. Such fragility fractures are associated with excess morbidity, mortality and substantial healthcare costs[38,125].

The pathophysiology underlying bone fragility in diabetes is multifactorial, with a complex interplay of molecular, hormonal, immune and genetic pathways[126] that may be unique to, or shared between both types of diabetes. T1D has a peak incidence in childhood and adolescence, where affected individuals have absolute insulin deficiency secondary to pancreatic beta-cell destruction. Low insulin and insulin-like growth factor-1 (IGF-1) levels affect bone turnover and impede bone formation during growth, thus reducing peak bone mass accumulation[127]. On the contrary, T2D impairs bone health at a later stage of disease, where reduced insulin secretion, glucose toxicity and pro-inflammatory cytokines converge to weaken bone microstructure and biomechanical properties. T2D is also underpinned by obesity and accelerated aging[128], whereby increased marrow adiposity, chronic inflammation[129] and sarcopaenia[130,131] have been implicated in musculoskeletal deterioration. Hyperglycaemia in uncontrolled T1D or T2D promotes the formation and deposition of advanced glycation end-products (AGEs) in bone matrix, which can compromise collagen properties and bone strength[125].

Discrepancies between bone mineral density (BMD) and fracture risk in both types of diabetes have been reported. Compared with individuals without diabetes, BMD appears to be lower in T1D, but normal or increased in T2D. Nevertheless, fracture risk remains disproportionately high in relation to BMD[37]. While traditional risk factors for fracture such as lower BMD, older age and female sex predict fractures in diabetes, this risk appears to be underestimated in fracture prediction algorithms, such as the IOF fracture risk assessment tool (FRAX®). Several clinical risk factors for fracture in individuals with diabetes have been identified, including poor glycaemic control and microvascular disease[33,124,132]. However, the independent contribution of female reproductive factors to fracture risk in this cohort is unclear.

Studies evaluating osteoporosis, falls and fractures across menopause in women with diabetes are scarce. Oestrogen deficiency, following menopause, is associated with a significant reduction in BMD[78,120]. In a large multi-ethnic cohort study, Greendale and colleagues[120] described a 10.6% and 9.1% 10-year cumulative postmenopausal BMD loss at the lumbar spine and femoral neck, respectively. Importantly, the majority of bone loss occurred in the one-year period before, through to two years following the final menstrual period.

A shorter duration of oestrogen exposure is an established risk factor for osteoporosis, and this may be pertinent in women with T1D, where later menarche and a trend toward earlier menopause has been reported[41,45,133]. Age of menarche and menopause in T2D, and relationships between reproductive lifespan and bone health in women with diabetes, have not been well explored. Only one study evaluated changes in BMD across the menopause transition in women with diabetes. Khalil and colleagues[121] prospectively followed women over a period of 8 years in the Study of Women Across the Nation (SWAN), and found that women with diabetes had significantly accelerated bone loss at the hip and a higher risk of fractures, compared with controls. In addition, the mean age of menopause was 49 years in women with diabetes, which was significantly earlier than that of controls (52 years).

Menopause represents an important transition in women where bone mass is rapidly lost. Coupled with the underlying mechanisms for skeletal fragility in diabetes, fracture risk may be markedly elevated during this time, which warrants consideration of screening and preventative strategies. In a large population-based cohort study using data from The Health Improvement Network, Weber and colleagues[33] showed that fracture risk in individuals with

T1D was increased throughout the lifespan, particularly after the age of 40 years. In age-stratified analyses, the adjusted risk of hip fracture appeared to be greatest in women with T1D aged 50 to 59 years, which may reflect the contribution of menopause to fracture risk. We hypothesize that female reproductive factors may be one of several important pathophysiological pathways involved in diabetes-related skeletal fragility. Therefore, we aimed to evaluate relationships between skeletal health and reproductive lifespan in women with T1D and T2D.

Methods

Study population

The Australian Longitudinal Study in Women's Health (ALSWH) was established in the early 1990s, after a call to develop and evaluate women's health policies. The research design consists of longitudinal evaluation of three age cohorts of women, using mailed surveys. The ALSWH examines the impact of demographic, social, physical, psychological and behavioural variables on women's health and well-being across the life course. At its inception in 1996, three cohorts of women of different ages, born in 1921 – 26, 1946 – 51 and 1973 – 78, were selected to participate in the study. A fourth cohort of women, born in 1989 – 95, was recruited in 2013. To date, nearly 60,000 women have participated in the ALSWH, making this the largest study of its kind in Australia. Further information on the ALSWH can be found elsewhere[134].

For the present study, we included Australian women of the mid-age cohort (born in 1946-51), which was specifically designed to examine the menopause transition, and the social and

personal changes of middle age. Women aged 45 to 50 years were randomly selected from the national health insurance scheme (Medicare) database to participate in the study. There was deliberate oversampling of women living in rural and remote areas (at twice the rate of women living in urban areas) to allow statistical comparisons of the health of city-dwelling women and those living in regional areas. Follow-up surveys were sent out in 1998, and at 3-yearly intervals, thereafter. Linked data to the Medicare Benefits Scheme and Pharmaceutical Benefits Scheme were subsequently available for selected states. The study period comprised six surveys administered over 15 years, from 1996-2000.

Diabetes status at baseline was obtained by self-report, using the questions 'Have you ever been told by a doctor that you have insulin-dependent (type 1) diabetes?' and 'Have you ever been told by a doctor that you have non-insulin-dependent (type 2) diabetes?'. Women who developed diabetes during follow-up were censored.

Demographic information was collected at baseline and at every survey, including age, alcohol use and cigarette smoking, highest level of education, and self-reported height and weight. Area of residence was classified by remoteness areas (major cities, inner regional, outer regional, remote and very remote), defined geographically using the Accessibility/ Remoteness Index of Australia (ARIA)[135], where a higher ARIA value indicates more remoteness.

Bone health, falls and physical activity

Women were asked from Survey 1 through 6 if they had ever been diagnosed with osteoporosis ('Have you ever been told by a doctor that you have osteoporosis?') For fracture, women were asked about any fractures within the last 12 months at Surveys 1, 2, 3, 5 and 6 ('In the last 12 months, have you had a broken bone (fracture)?'). Falls data was gathered from Survey 4 and onwards. Women were asked if they had 'experienced a fall to the ground (not including stumbles or trips)' and if they had 'been injured as a result of a fall' in the past 12 months. Physical activity information was collected at every survey, where women were asked about the time spent walking (brisk), on moderate and vigorous leisure activities, in a given week. Indirect estimates of energy expenditure were derived in the form of metabolic equivalent (MET) minutes per week, based on calculations on the amount of time spent doing each activity by the allocated MET values for each category[136]. The threshold used for 'adequate physical activity for health benefit' was equal to or greater than 600 metabolic minutes per week (equivalent to 30 minutes of moderate activity five days per week), based on the World Health Organization recommendations[136,137].

Reproductive factors

Women were asked about their menopause status at every survey and age of menopause was asked in Surveys 2 to 6. Those who reported ever having a hysterectomy or both ovaries removed, were classified as having undergone surgical menopause. Premature ovarian insufficiency (POI) was defined as cessation of menses before age 40, and early menopause was defined as cessation of menses before age 45. Information on menopausal hormone therapy (MHT) use and duration was collected at all surveys, and age of menarche was collected in Survey 2 only.

Statistical analyses

Stata/IC 15.1 for Windows was used for all analyses. Demographic data of women with and without diabetes were summarized and compared as three groups (T1D, T2D and controls). Continuous and categorical data were presented as means (± standard deviation) and percentages and analyzed using the student's t-test and chi-squared test, respectively.

Random effects logistic regression was used to analyze the relationship between incident fracture and new cases of osteoporosis with diabetes status. Univariable logistic regression was first performed, with variables retained at a significance level of $p < 0.1$. The final multivariable logistic regression model incorporated statistically and clinically significant variables. In addition, bootstrapping was performed with 1000 repetitions at 95% resampling of the original dataset. Statistical significance was defined as $p<0.05$.

Results

Baseline demographics

11,313 women were eligible for inclusion into the study (T1D=107; T2D=332; controls=10,874). Baseline demographic characteristics across the three groups are summarized in Table 1. At Survey 1, the mean age of participants was 47 years, which was similar across all groups (47.3±1.5 vs. 47.1±3.0 vs. 47.1±1.5, p=0.48). Mean BMI was significantly higher in women with T1D and T2D, compared with controls (29.1±7.3 and 30.5±7.5 vs. 25.5±4.8 kg/m^2, p<0.001). There were no significant differences in the proportion of women who were current smokers, ex-smokers or never-smokers in each group, although alcohol use was higher in controls (39.6% vs. 29.9% vs. 55.6%, p<0.001). A large

majority of participants resided in urban areas, with approximately 5 percent of women living in rural and remote areas (5.6% vs. 6.9% vs. 4.9%, p=0.24). Significantly less women with T1D and T2D completed tertiary education, compared to their non-diabetic counterparts (27.6% and 27.2% vs. 34.9%, p=0.006). A significantly higher proportion of women with T1D and T2D were of Aboriginal and Torres Strait Islander status (4.8% vs. 3.6% vs. 0.7%, p<0.001), compared with their non-diabetic counterparts. More women with T2D had concomitant cardiovascular and respiratory comorbidities, compared to those with T1D and without diabetes. Overall, the attrition over 15 years was 20%, where the remaining number of women at Survey 6 with T1D, T2D and no diabetes were 67,218 and 8,821, respectively.

Bone health outcomes, falls and physical activity

At baseline, there was no significant difference in self-reported osteoporosis prevalence between groups, although fractures were significantly more prevalent in women with T1D and T2D, compared to controls (15.1% and 18.5% vs. 7.6%, p<0.001). Over 15 years, 885 new cases of osteoporosis (T1D, n = 22; T2D, n = 20; controls, n = 843) and 1,099 incident fractures (T1D, n = 19; T2D, n = 47; controls, n = 1,033) were reported. 6,730 falls occurred from 2004 to 2010. Of these, 3,392 women had a fall-related injury. Falls were reported by 58.8% and 52.4% of women in the T1D and T2D group, respectively, which was significantly higher compared with controls (p=0.004). Significantly more women with T1D and T2D reported recurrent falls (32.9% and 34.3% vs. 27.0%, p =0.02) and fall-related injuries (35.3% and 32.3% vs. 26.4%, p =0.018), compared with controls. In terms of average MET minutes per week, all three groups met the recommendation for adequate physical activity, although this was significantly lower in women with T1D, compared with women with T2D and controls (834.9±95.6 vs 946.7±51.7 and 1074.2±10.2 minutes, p<0.001). Furthermore, a significantly lower proportion of women with T1D met physical activity

recommendations, compared with T2D and controls (47.6% vs. 59.0% and 61.8%, p<0.007) [Table 2].

Reproductive outcomes

Women with T1D had significantly later menarche (13.1±1.7 vs. 12.5±1.8 and 12.9±1.9 years, p<0.001) and an earlier age at menopause (47.7±3.4 vs. 49.9±4.8 and 50.3±3.7 years, p<0.001), compared with women with T2D and controls. Consequently, reproductive lifespan was approximately 2.5 years shorter in women with T1D (34.8±3.7 vs. 37.1±5.0 and 37.4±4.0 years, p<0.001). A significantly higher proportion of women with T2D reported POI, compared with T1D and controls (4.2 vs. 1.5 and 1.2%, p=0.003). There were no differences observed in the proportion of women who had early or surgical menopause, or MHT use and duration of use between the three groups (Table 3).

Regression analyses

T1D, but not T2D, was associated with a two-fold increased risk of self-reported osteoporosis on univariable regression analysis (OR 2.30, 95% CI 1.53 – 3.45, p<0.001). After adjustment, T1D and MHT use were associated with a 2.5- and 1.5-fold increased risk of osteoporosis, respectively (T1D: OR 2.66, 95% CI 1.27 – 5.58, p<0.001; MHT use: OR 1.44, 95% CI 1.19 – 1.75, p<0.001). Higher age appeared to be protective for osteoporosis (OR 0.96, 95% CI 0.94 – 0.98, p<0.001) [Table 4].

In univariable regression analyses, both T1D and T2D were associated with a two-fold increased risk of fracture (T1D: OR 2.28, 95% CI 1.53 – 3.40, p<0.001; T2D: OR 2.40, 95%

CI 1.90 – 3.03, p<0.001). After adjustment, T2D, but not T1D, was associated with a more than 2-fold greater risk of fracture, as was a history of falls (T2D: OR 2.49, 95% CI 1.64 – 3.77, p<0.001; falls: OR 2.17, 95% CI 1.80 – 2.61, p<0.001). A longer reproductive lifespan and higher age were both protective for fracture (reproductive lifespan [per 5 years]: OR 0.88, 95% CI 0.80 – 0.98, p=0.02; age: OR 0.98, 95% CI 0.96 – 0.99, p=0.005) [Table 5].

Discussion

Across the menopause transition, women with T1D and T2D exhibit increased falls, osteoporosis and fracture, compared with their non-diabetic counterparts. Reproductive lifespan was reduced, with later menarche and earlier menopause, in women with T1D. A shorter reproductive lifespan, leading to reduced estrogen exposure, alongside increased falls, may increase fracture risk in T1D, while falls are also likely contributors in T2D, presenting opportunities for prevention.

T1D and T2D are associated with increased falls risk in older persons, via direct and indirect mechanisms, such as hypoglycaemia[106], retinopathy, neuropathy and sarcopaenia[138,139]. A 64% increase in falls was reported in a meta-analysis of older adults with diabetes, predominantly over the age of 70 years[140]. Impaired balance and low muscle strength are consistent findings on physical performance testing in individuals with diabetes[29,83,141] and contribute to falls risk. In the Study of Osteoporotic Fractures, poorer balance was partly accountable for increased falls in older women with diabetes. Although this study was not able to differentiate between diabetes types the odds ratio for falls was 1.68 for those with non-insulin treated diabetes, and 2.78 for women on insulin therapy[142]. In our study, we observed that more than half and a third of women with T1D and T2D reported falls and

injurious falls, respectively, which is unexpectedly high for a cohort of relatively younger women, who were aged between 60 to 65 years at the end of follow-up. Recurrent injuries from falls were higher in women with T2D, compared to women with T1D and controls. Notably, comorbidities known to confer an increased risk of falls, such as cardiovascular and respiratory conditions[143-145], were significantly higher in women with T2D in this study.

Physical activity was significantly lower in women with T1D, with less than half of women in the T1D group achieving the recommended 600 MET minutes per week. Data on physical activity levels in older adults with T1D are limited, although a large study of young to middle-aged adults with T1D observed that only 33% of this cohort met the recommended physical activity guidelines[146]. Microvascular complications, such as retinopathy and neuropathy, a higher risk of hypoglycaemia on insulin therapy and depressive symptoms related to chronic disease[146,147] may prevent engagement in physical activity or exercise programs. However, there is evidence that increased physical activity and exercise intervention may improve physical function to reduce falls-related injuries and fractures in older adults[148-150]. Overall, reduced physical activity in women with T1D and the presence of cardiorespiratory comorbidities in women with T2D, may in part account for the greater falls risk in these groups.

Our findings of an increased osteoporosis risk in women with T1D is supported by earlier studies reporting lower BMD in individuals with T1D, compared with non-diabetic individuals[37,151]. We did not observe a relationship between T2D and osteoporosis, which is consistent with established findings of higher BMD in individuals with T2D[152]. Earlier studies have reported impaired bone quality and microarchitecture in T2D[153-155], which may

account for increased skeletal fragility, despite preserved BMD. We observed paradoxical associations of MHT use and older age with increased and reduced osteoporosis risk, respectively. Women at greater risk for osteoporosis are more likely to be prescribed MHT by health practitioners, so that MHT use may be a surrogate marker for at-risk women. The association of older age and reduction in osteoporosis risk may be due to survivor effect. Notably, while statistically significant, the risk reduction with higher age is minor, and therefore unlikely to be clinically significant.

In univariate analyses, both T1D and T2D were significantly associated with increased fracture risk. However, only T2D status remained significantly associated with fracture risk in the multivariable regression model, with the effect size observed being comparable to previous studies[38]. A longer reproductive lifespan appeared to be protective for fractures, and conversely, a history of falls was associated with increased fracture risk. After adjustment for reproductive lifespan and falls, we observed an attenuation in the association between T1D status and fracture, suggesting that these factors are likely to mediate fracture risk in women with T1D.

Earlier menopause is an independent risk factor for both reduced BMD and fracture[156-158]. With later menarche and earlier menopause, women with T1D had a significantly reduced reproductive lifespan, while those with T2D appeared to attain menarche and menopause at similar ages to their non-diabetic counterparts. Our results are consistent with the only other study to evaluate reproductive factors and its relationship with bone health in women with diabetes, by Khalil and colleagues[121], in which women with diabetes reached menopause three years earlier than non-diabetic controls. Notably, we have differentiated between risk

factors in T1D and T2D, whereas this distinction was not possible in the aforementioned study.

A trend toward earlier menopause was observed in a study of women with T1D and their family members[133], where those with T1D were more likely to have later menarche and earlier menopause, compared to their non-diabetic sisters and controls, resulting in a 6-year reduction of their reproductive lifespan. The age of menopause in women with T2D is less well defined, given the typical post-menopausal onset of T2D, in the face of anthropometric and metabolic changes that occur with oestrogen deficiency. Early menopause and POI have been identified as risk factors for developing T2D[159,160]. We observed a higher proportion of women with T2D reporting POI, although it is unclear whether POI predisposes to T2D development at a younger age, or if younger onset of T2D affects ovarian reserve.

There were several limitations to our study. The classification of diabetes status and several outcomes of interest were determined by self-report, which is subject to a degree of misclassification bias. The Women's Health Initiative, a large epidemiological study of post-menopausal women conducted around the same time period as our study, showed that diabetes self-report was concordant with fasting glucose measurements and medication inventories[161]. Also, Peeters and colleagues[162] examined the validity of self-reported osteoporosis in this ALSWH cohort using medication information and pharmaceutical reimbursement claims, and found that concurrent validity was moderate to good for self-reported prevalent osteoporosis, but poor to moderate for self-reported incident osteoporosis. In addition, self-reported prevalent and incident osteoporosis was significantly associated with at least 3 out of 5 characteristics selected for construct validity (fracture, reduced physical functioning and lower weight). The validity of self-reported fractures in

postmenopausal women is generally high[163], especially for hip, wrist and humeral fractures[164,165]. Information on fracture sites, mechanisms, BMD data, biochemical and/or clinical data pertaining to diabetes duration, glycaemic control and complications, was not available. Apart from MHT, we were unable to determine the use of anti-osteoporosis medications.

The strengths of this study include the longitudinal design and a large sample size with relatively low attrition, over 15 years. Another strength was the comprehensive variables available in the dataset, including reproductive lifespan, physical activity and falls history, which enabled effect size estimates (odds ratios) to be calculated adjusting for known confounders. Bootstrapping was also done, supporting the robust results. Fractures are uncommon events in such a relatively young cohort of women; however, the timing of this study in relation to age, allowed us to study of bone health outcomes through the menopause transition and beyond. We were able to collect comprehensive health and lifestyle information in unselected community-dwellers thought to be representative of women in Australia.

Conclusions

Across the menopause transition, women with T1D and T2D have increased BMI, falls and falls-related injuries, which are risk factors for adverse musculoskeletal outcomes. T1D and T2D were independently associated with increased risk of incident osteoporosis and fracture, respectively. Reproductive lifespan was significantly reduced in women with T1D and may act as an intermediary between T1D status and fracture risk. Indeed, potential earlier menopause in women with T1D can predispose to lower BMD and skeletal fragility.

Clinicians should be aware of the shorter reproductive lifespan and potential earlier age of menopause in women with T1D, and initiate assessment and education on bone health in those affected. Modifiable risk factors, such as falls and hypoglycaemia, should be optimized for fracture prevention in women with diabetes. Further studies of reproductive factors in diabetes may clarify the complex fracture mechanisms in this cohort. Further longitudinal studies on BMD changes and fracture risk in women with T1D and T2D are needed to guide optimal timing of bone health assessment and interventions in these groups.

Acknowledgements

The research on which this paper is based, was conducted as part of the Australian Longitudinal Study on Women's Health, by the University of Queensland and the University of Newcastle. We are grateful to the Australian Government Department of Health for funding and to the women who provided the survey data.

Tables & Figures

	Controls (n=10874)	T1D (n=107)	T2D (n=332)	p-value
Age, years	47.1 ± 1.5	47.3 ± 1.5	47.1 ± 3.0	0.48
Mean follow-up duration, years	13.6 ± 3.3	12.1 ± 4.3	11.7 ± 5.1*	**<0.001**
BMI kg/m^2	25.5 ± 4.8	29.1 ± 7.5*	30.5 ± 7.3*	**<0.001**
Smoking (%)				0.12
Never smoker	54.4	57.4	53.5	
Current smoker	28.4	26.7	24.2	
Ex-smoker	17.1	15.8	22.3	
Alcohol use (%)	55.6	39.6	29.9	**<0.001**
Aboriginal/Torres Strait Islander (%)	0.7	4.8	3.6	**<0.001**
Living in remote/very remote areas^ (%)	4.9	5.6	6.9	0.24
Tertiary education (%)	34.9	27.6	27.2	**0.006**
Heart disease (%)	1.9	3.7	5.8*	**<0.001**
Hypertension (%)	19.9	17.9	46.1*	**<0.001**
Stroke (%)	0.7	0.9	2.7*	**<0.001**
COPD/emphysema (%)	17.6	10.3	21.1*	**0.04**
Asthma (%)	15.2	5.6	22.4*	**<0.001**
Iron deficiency (%)	31.6	26.2	31.4	0.49

Table 1. Cohort demographics
*Difference between groups $p<0.05$ on post hoc analyses with Bonferroni correction
^Defined using the Accessibility/Remoteness Index of Australia

	Controls (n=10874)	T1D (n=107)	T2D (n=332)	p-value
Fracture at baseline (%)	7.6	15.1*	18.5*	**<0.001**
Osteoporosis at baseline (%)	3.5	2.8	5.4	0.15
Ever had a fall (%)	45.4	58.8*	52.4*	**0.004**

Recurrent falls (%)	27.0	32.9*	34.3*	**0.02**
Ever had an injurious fall (%)	26.4	35.3*	32.3*	**0.018**
Recurrent injurious falls (%)	6.0	7.9	10.4*	**0.02**
Average metabolic minutes (min/week)	1074.2±10.2	834.9±95.6*	946.7±51.7	**<0.001**
MET min > 600/week (%)	61.8	47.6	59.0	**0.007**

Table 2. Musculoskeletal outcomes
*Difference between groups p<0.05 on post hoc analysis

	Controls (n=10874)	T1D (n=107)	T2D (n=332)	p-value
Age at menarche, years	12.9 ± 1.9	13.1 ± 1.8*	12.5 ± 1.7	**<0.001**
Age at first pregnancy, years	23.0 ± 4.5	22.2 ± 4.8	21.9 ± 4.4*	**<0.001**
Mean no. of livebirths	2.4 ± 1.1	2.4 ± 1.0*	2.5 ± 1.2*	**0.03**
Age at menopause, years	50.3 ± 3.7	47.7 ± 3.4*	49.9 ± 4.8	**<0.001**
Reproductive lifespan[#], years	37.4 ± 4.0	34.8 ± 3.7*	37.1 ± 5.0	**<0.001**
Premature ovarian insufficiency[1] (%)	1.2	1.5	4.2*	**0.003**
Early menopause[2] (%)	5.1	10.6	6.5	0.10
Surgical menopause (%)	25.7	23.8	25.9	0.76
MHT, menopausal hormone therapy				0.94
Never users	73.6	72.0	73.2	
<10 years	25.2	26.2	25.9	
>10 years	1.2	1.9	0.9	

Table 3. Reproductive parameters
#Reproductive lifespan: period between menarche and menopause
*Difference between groups p<0.05 on post hoc analyses with Bonferroni correction
[1] Cessation of menses < 40 yrs; [2] Cessation of menses < 45 years

	Odds ratio	95% CI	p-value
T1D^	2.66	1.27 – 5.59	0.01
T2D^	1.03	0.52 – 2.01	0.94
Reproductive lifespan (per 5 years)^	1.05	0.93 – 1.19	0.39
Age^	0.96	0.94 – 0.98	<0.001
MHT use^	1.44	1.19 – 1.75	<0.001

Table 4. Random effects logistic regression for new cases of osteoporosis.
^Adjusted for diabetes status, BMI, age, falls history, reproductive lifespan and MHT use

	Odds ratio	95% CI	p-value
T1D^	1.73	0.82 – 3.68	0.15
T2D^	2.49	1.64 – 3.77	<0.001
Reproductive lifespan (per 5 years)^	0.88	0.80 – 0.98	0.019
Age^	0.98	0.96 – 0.99	0.005
Falls history^	2.16	1.80 – 2.61	<0.001

Table 5. Random effects logistic regression for incident fracture.
^Adjusted for diabetes status, BMI, age, falls history, reproductive lifespan and MHT use

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4.2 SUMMARY & CONCLUSIONS

Chapter 4.1 utilised data from the ALSWH cohort of women born in 1946 – 1951, designed to assess physical and emotional health and health behaviours with changes of menopause, in middle-aged women. Over a period of 15 years (1996 – 2000), I showed that women with T1D and T2D have a greater risk of osteoporosis, fractures and falls during the menopause transition. With later menarche and earlier menopause, reproductive lifespan was significantly reduced in women with T1D, compared with those with T2D and controls. BMI was increased in both groups of women with T1D and T2D, and physical activity was significantly lower in the T1D group.

T1D was associated with an increased risk of osteoporosis, supported by earlier studies reporting lower BMD in this cohort[37,151]. The association between T1D and fracture was attenuated after adjustment for reproductive lifespan and falls, suggesting that these factors are potential intermediaries in T1D-related skeletal fragility. The key limitations in this study were the lack of clinical information for T1D duration and control, fracture sites, mechanisms and BMD measurements, as well as the use of self-reported outcomes for T1D, fracture and osteoporosis, which may increase the risk for misclassification. However, the findings from this study are consistent with an earlier cohort study by Khalil and colleagues[121], providing basis for reduced oestrogen exposure due to a shorter reproductive lifespan, as a potential mechanism of skeletal fragility in T1D. In addition, this study provides an Australian perspective on reproductive and bone health, in women with diabetes. Diabetes confers a higher risk of falls in older adults, where poorer balance and insulin use contributes to increased falls[142]. The menopause transition is associated with a heightened risk of osteoporosis, fractures and falls in women with T1D, and consideration should be given to screen and optimise risk factors in women at high risk of skeletal events.

5. CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

Bone health in T1D

T1D confers an increased risk of fractures throughout the lifespan. There is a substantially elevated risk of hip fractures, which is disproportionate to BMD[37]. The pathogenesis of skeletal fragility in T1D is multifactorial, with the interplay of genetic, molecular, cellular and clinical factors, which are still not well understood. Mouse models and limited human studies of bone histomorphometry in T1D have provided greater insight into the pathways of skeletal fragility. In addition to reduced bone mass, bone microstructure is also impaired in T1D, where alterations in the cortex and trabecular microarchitecture can further compromise bone biomechanical properties. There is further suggestion that diabetic bone disease may be an extension of diabetic microangiopathy[102]. However, there is some debate about microvascular complications being a proxy for poorer metabolic control and a longer duration of diabetes, so that the independent effect of diabetic microangiopathy is unclear. Nevertheless, some cross-sectional studies have reported associations between low BMD and the presence of retinopathy[166-168], nephropathy[166-169] and neuropathy[168,170-172], which are independent of T1D duration or glycaemic control.

Deficits in volumetric BMD and bone microarchitecture have been reported in patients with T1D and microvascular complications. Shanbhogue and colleagues observed a reduction in cortical and trabecular volumetric BMD at the radius and tibia, explained by cortical thinning and reduced trabecular numbers, respectively[168]. These reductions remained apparent after adjustments for confounders, such as T1D duration and glycaemic control. Likewise, Abdalrahaman and colleagues also observed similar trabecular compartment deficits in patients with T1D and retinopathy, compared with those with T1D without complications and healthy controls[86]. Large population-based cohort studies[100,101] have demonstrated that the

presence of microvascular disease increases fracture risk in excess of having T1D alone, although the lack of information on glycaemic control raises the possibility of poor metabolic control as a potential confounder. Several other confounders may alter the association between microvascular disease and fracture in T1D, such as a greater predisposition for falls, related to impaired vision and neuropathy. The association between microvascular disease and fracture risk, independent of falls, is still unknown. Overall, it appears that the risk of fracture is higher in individuals with chronic poor metabolic control and/or microvascular complications, in addition to the presence of other risk factors for osteoporosis.

Despite the increasing recognition of the association between T1D and fragility fractures, there is a distinct lack of guidelines for fracture risk assessment and management in this cohort. Although fractures can occur at any age, there is no consensus regarding the timing and mode of bone health assessment. Although BMD assessment by DXA carries a low radiation risk, this may be challenging in children and young adults with T1D, where the validity of BMD interpretation prior to accrual of peak bone mass is controversial. In general, the absolute fracture risk in young adults is low, and the yield of BMD testing in a young adult population with T1D is currently unclear. The use of fracture prediction calculators, such as FRAX®, which utilises BMD in its algorithm, appear to underestimate fracture risk in individuals with diabetes[173], although modifications in FRAX have been proposed to overcome this[40]. Furthermore, the use of FRAX® in adults under the age of 40 has not been validated. Therefore, evaluation of bone health and fracture risk in young adults with T1D remains an ongoing challenge.

Novel imaging modalities, such as TBS and HRp-QCT, have allowed for non-invasive, indirect quantification of bone microarchitecture, and may be useful adjuncts to BMD to identify those at greater risk of fracture. TBS is now increasingly more accessible as a diagnostic tool and can be incorporated into FRAX to enhance fracture prediction, with or

without BMD[90,174,175]. However, HRp-QCT is only available in three centres in Australia, and is currently utilised in a research setting, although age-, sex- and site-specific reference ranges have been developed[176], which may broaden its application in clinical practice. Finally, the management of osteoporosis in this cohort is contentious, with limited information regarding the efficacy of common anti-osteoporosis medications in adults with T1D. The effect of anti-resorptive medications, such as bisphosphonates and denosumab in T1D, a condition associated with low bone turnover, is unknown, and therefore poses a therapeutic dilemma. In this unique context of skeletal fragility, anabolic agents such as teriparatide and romosuzumab, may be of potential benefit[173]. However, now that the problem and potential antecedents are identified, in addition to the areas my work has contributed to, it is clear that more research is needed to drive practice and inform guidelines.

Overall, despite the known increased risk of fracture in T1D across the lifespan, there is currently insufficient basis to recommend BMD testing for all individuals with T1D. However, strategies to reduce fracture risk appear underutilised in this population, possibly related to challenges of identifying high-risk patients and concerns regarding effective treatments for prevention[177]. Screening for risk factors known to confer a greater risk of skeletal fragility, such as prior fracture, poor glycaemic control, microvascular disease, high falls risk, and/or concomitant autoimmune disease, at least annually, may help identify a select group of higher-risk individuals who may benefit from BMD testing. Optimisation of risk factors for fracture, such as falls prevention and assessment of microvascular complications of diabetes, are essential in older adults with T1D. The predilection for hip fractures in adults with T1D remains undifferentiated, and imaging modalities, such as hip structural analysis[178], may provide more insights into fracture mechanisms at this site. Further prospective studies evaluating the relationship between clinical risk factors, BMD and/or bone microarchitecture and fracture outcomes, are needed to influence policies and guidelines for screening.

Reproductive health in T1D

T1D is associated with a spectrum of reproductive disorders that can manifest across the reproductive lifespan, starting from puberty and menarche, and ending at menopause. The characteristics of menstrual and reproductive disorders in T1D has evolved tremendously since the introduction of insulin therapy. Perturbations of the HPO axis in the face of metabolic disruption and poor glycaemic control have been described previously[41,179], resulting in pubertal and menarchal delay, amenorrhea and infertility. Insulin therapy, and intensification of insulin regimens over the last few decades, have afforded better glycaemic control and improved the disease course in individuals with T1D. While pubertal and/or menarchal delays are now rare in girls with T1D, menstrual irregularity and reproductive dysfunction continue to be a feature. However, PCOS, a condition typically associated with insulin resistance, obesity and the metabolic syndrome, is now emerging in T1D[60], which may portend increased reproductive problems, such as infertility, in addition to substantial cardio-metabolic and psychological morbidity. The exogenous hyperinsulinaemia associated with subcutaneous insulin administration, leading to increased ovarian follicle stimulation and androgen production, has been implicated as a key mechanism for PCOS development[61,62]. Furthermore, hyperinsulinaemia predisposes to weight gain, which can perpetuate a vicious cycle of insulin resistance, increasing insulin doses and weight gain. The rise of obesity rates in T1D is concerning, where the prevalence of overweight and obesity in this cohort approximates 50%. Notably, this prevalence of obesity increased by 7-fold, from the 1980s to mid-2000[115], coinciding with the increased uptake of intensive insulin therapy. There is evidence to show that intensification of insulin therapy is associated with excess weight gain, central obesity and dyslipidaemia[112], which negates the benefits of good glycaemic control.

The cause for purported earlier menopause in T1D is not completely understood, although the mechanisms are likely to be diverse, as in the case of skeletal fragility. Amongst the postulated theories of autoimmune oophoritis and ovarian glucotoxicity, consideration should also be given to exogenous hyperinsulinaemia as a cause of accelerated ovarian follicle

depletion. Menopause is associated with adverse metabolic changes and reduced insulin sensitivity, which can exacerbate dysglycaemia in women with pre-existing diabetes. Evidence suggests that the use of MHT improves the metabolic profile and glucose homeostasis in women with T2D[180,181], although this has not been studied in women with T1D. A Cochrane review in 2013 concluded there was insufficient evidence to make recommendations for MHT use in women with T1D, after identifying only one small RCT that included women with T1D[182]. Furthermore, many of these studies were not powered to assess clinical outcomes and adverse effects. The menopause transition is also associated with a significant loss in bone mass, representing a period of increased fracture risk, in women who are already predisposed to fracture.

Challenges and future directions

The repercussions of T1D and insulin therapy on the bone and reproductive systems across the lifespan have been discussed extensively throughout this thesis. Skeletal fragility and reproductive dysfunction are emerging complications of T1D, which contributes to excess morbidity and healthcare costs. The propensity for hip fracture in T1D is a consistent observation amongst several large observational cohort studies across different countries, deserving although the mechanisms by which this occurs is currently unclear. Importantly, my findings from the systematic review and meta-analysis in Chapter 1.1.4 showed that the risk of hip fracture in young adults is four-fold greater than that of non-diabetic controls, with a slightly increased risk seen in females with T1D. Presently, there are no guidelines in place to assist clinicians in identifying and managing at-risk patients. Fracture risk stratification in individuals with T1D is further complicated by the discrepantly high fracture risk for a given BMD, and the use of adjunctive imaging techniques and bone biomarkers are still under study. Clinical risk factors, such as chronic poor glycaemic control[97,98], recurrent hypoglycaemia[84], microvascular disease and falls, may help identify those at greater risk of

fracture. Co-existent causes of secondary osteoporosis, such as coeliac disease[33,183], hyperthyroidism and POI, should be screened for and treated. My cross-sectional study in Chapter 2.1.1 provides new insights on the associations between concomitant coeliac disease, hypoglycaemia and prevalent fracture, and contributes to the small handful of studies examining skeletal outcomes, glycaemic control and microvascular complications in a young adult T1D population. The decision to perform DXA in younger adults, particularly in females of child-bearing age, should be at the discretion of the clinician, taking into account accompanying risk factors and fracture history. In Australia, BMD testing is currently indicated for individuals over the age of 50 with a minimal trauma fracture, or if risk factors for secondary osteoporosis are present. Screening for osteoporosis in males and females without fracture is recommended after the age of 70. My findings of earlier menopause in women with T1D, in Chapter 4.1, is consistent with results from an earlier cohort study of multiethnic women in the UK[121], which reported that menopause occurred on average 3 years earlier in women with T1D, in association with increased fractures during the menopause transition. My study contributes to the limited data of reproductive information in women with T1D, and provides credence to the growing evidence of a shorter reproductive lifespan in this cohort, with adverse effects on the skeleton. Therefore, a proposal to lower the age threshold for screening may be appropriate in this context, to ensure timely identification and treatment of affected women. Further studies evaluating the changes in BMD and fracture risk in women with T1D across the menopause transition and beyond, can guide the timing of BMD testing. Another pertinent finding in Chapter 4.1 was the significantly increased frequency of falls in post-menopausal women with T1D, which may be another intermediary between T1D and fracture risk. Optimization of risk factors for falls in older women with diabetes, such as hypoglycaemia prevention, may help mitigate fracture risk.

In Chapter 1.2.7, I led and performed an extensive literature review of reproductive health across the lifespan in women with diabetes, detailing the mechanisms and evolution of

menstrual and reproductive disturbance in this cohort, while introducing an important paradigm shift of diabetes as a reproductive and metabolic disorder in women[45]. The persistence of menstrual and reproductive disorders despite improved glycaemic control is of concern, particularly in the setting of increased obesity rates in individuals with T1D. Chapter 3.1 details the association between women with T1D and an increased risk of menstrual irregularity and PCOS, as well as the strikingly high prevalence of obesity in a contemporary T1D cohort of reproductive age. Through this large Australian population-based study, I was able to demonstrate the excessive risks of menstrual dysfunction and PCOS in T1D, compared with the general female population, which was independent of BMI. Obesity contributes to metabolic and reproductive dysfunction in T1D, and should be aggressively managed in young women. Interventions incorporating healthy lifestyle and prevention of excessive weight gain may improve metabolic control and reproductive outcomes.

Reproductive dysfunction is under-recognised and not adequately addressed in the clinical setting. Kohn and colleagues[184] demonstrated that a high proportion of healthcare providers were reluctant to discuss contraception and preconception care in young women with T1D, citing insufficient time and inadequate subject knowledge as barriers. Greater awareness of the rising prevalence of PCOS in the context of intensive insulin therapy is needed amongst clinicians and patients alike, and consideration given to screen for ‘secondary PCOS’, where clinically indicated. Women with T1D are at risk of a shortened reproductive lifespan, which may have important implications on cardiovascular and bone health. Furthermore, the impact of concomitant PCOS and T1D on long-term cardiometabolic outcomes is not known. Further research evaluating therapeutic strategies, such as the role of metformin as an insulin sensitiser in women with T1D and PCOS, and the efficacy and safety profile of MHT in women with T1D, is much needed.

Overall, the studies presented within this thesis have extensively discussed and highlighted the impact of T1D and repercussions of contemporary therapy, on skeletal and reproductive

outcomes. The key learnings and recommendations from my thesis are summarised in Table 4. Moreover, the latter two studies in Chapter 3.1 and 4.1 provide an Australian perspective of menstrual and reproductive problems in women with T1D, which has not previously been described. The findings of increased fracture hip risk in younger adults with T1D, and a greater risk of osteoporosis in women with T1D across the menopause transition, demonstrate that conditions typically associated with old age, such as osteoporosis and hip fractures, manifest at an unusually early age in individuals with T1D. Earlier menopause in women with T1D may play a role in the pathogenesis of diabetes-related skeletal fragility.

Finally, the rising prevalence of PCOS appears to occur in parallel with increasing obesity prevalence in young women with T1D, necessitating strategies in place to manage weight gain and attendant cardiometabolic risks, while balancing glycaemic control. There appears to be an interplay of skeletal and reproductive factors in T1D, which may be an extension of the spectrum of metabolic complications in this cohort. Increased awareness, ongoing research and evidence-based guidelines to optimise women's reproductive, bone and metabolic health in the unique context of T1D, should be a priority. In my postdoctoral studies, I intend to progress this work by evaluating the relationship between androgen profile, menstrual disorders and metabolic characteristics in young women with T1D, with the aim of increasing awareness and improving the identification of hyperandrogenaemia and/or PCOS in this cohort.

	Key findings from thesis	Recommendations	Future research focus areas
Bone health	• Increased skeletal fragility in individuals with T1D; 4-fold greater risk of hip fracture in young to middle-aged adults with T1D, compared to controls	• Consider DXA in individuals identified to be at high risk of fracture (prior fracture, poor glycaemic control, microvascular disease, high falls risk, concomitant autoimmune disease) • If available, TBS and HR-pQCT may be a useful adjunct to DXA in fracture risk stratification • Optimisation of risk factors for falls/fracture	• Timing of and optimal imaging methods for bone health assessment, particularly in a young adult cohort • Hip structural analysis in T1D • Management of osteoporosis in T1D
	• Fracture risk increased in young adults with T1D and concomitant coeliac disease	• Increased hypoglycaemia and vitamin D deficiency is a feature in those with T1D and coeliac disease, and should be managed • DXA may be considered	• Evaluation of fracture risk in individuals with T1D and other autoimmune conditions, eg. autoimmune hyperthyroidism
Bone and reproductive health	• Shorter reproductive lifespan and higher falls risk in postmenopausal women with T1D may mediate bone fragility	• Consider lowering age threshold for BMD screening in postmenopausal women with T1D • Optimise risk factors for falls (eg. hypoglycaemia, gait aids etc)	• Effect of earlier menopause on fracture risk and bone health • Efficacy and safety of MHT in women with T1D
Reproductive health	• Menstrual disorders highly prevalent in young women with T1D; risk of menstrual irregularity and PCOS 1.5- and 2.5-fold greater in women with T1D compared to controls • Rising prevalence of PCOS in T1D may be partly attributable to intensive insulin therapy and increasing prevalence of obesity in this cohort	• Screen reproductive-aged women for menstrual disturbance and monitor weight gain; consider screening for PCOS in those at high risk (overweight/obese, menstrual irregularity, clinical hyper-androgenaemia) • Monitor metabolic risk factors in those with PCOS and T1D • Consider lifestyle strategies and pharmacotherapy (eg. metformin) to manage overweight and obesity	• Genetic and metabolic characterisation of T1D and PCOS • Optimal treatment of PCOS in T1D • Long-term metabolic outcomes in women with T1D and PCOS

Table 4. Summary of key findings and recommendations for optimising bone and reproductive health in individuals with T1D

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APPENDIX: OTHER PUBLICATIONS PRODUCED DURING CANDIDATURE

Naderpoor N, Garad R, **Thong EP**, Teede HJ. *PCOS & Diabetes: New Management Guidelines. Diabetes Management Journal*, Nov 2018

FEATURE

PCOS & DIABETES:
NEW MANAGEMENT GUIDELINES

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting reproductive aged women[1] and is associated with a 4-times higher rate of T2D. The **Australian authors** of the new international PCOS guidelines[20] summarise what clinicians should look for, and what can be done about PCOS when it is found.

PCOS is a prevalent, complex condition with a heterogenous range of reproductive, metabolic and psychological symptoms. The condition is undiagnosed in up to 70% of affected women and key features such as a psychological burden and metabolic risks are under-recognised. Women with PCOS report dissatisfaction with diagnosis experiences, lack of quality information and inconsistent management practices. To achieve optimal health outcomes for women with PCOS a holistic, person-centered approach implemented within a biopsychosocial model of care is recommended.

EPIDEMIOLOGY

PCOS is an ancient, complex genetic disorder[2] with a genomic footprint going back 50,000 years.[3] It is postulated that the genetic regulation of fertility may have been advantageous in the feast or famine setting.[4] However, in the current obesogenic environment PCOS has detrimental short and long-term health impacts.

Intrauterine events may predispose to this condition, including hyperandrogenaemia in the uterus or excess maternal hormones (including anti-müllerian hormone) acting on the developing endocrine system.[5] Excess weight gain is significant in translating a predisposition to PCOS into clinical manifestation.[6]

PCOS is increasingly recognised as a condition-affecting women across the life span with hyperandrogenic symptoms (acne, hirsutism) most evident in adolescents and increased metabolic risks (diabetes, central obesity and CVD risk factors) more prominent later in life.

The prevalence of PCOS in reproductive aged women ranges from 8–13% depending on the criteria used to diagnose the condition.[7-9] Women at higher risk of manifesting PCOS include those who are overweight, Aboriginal and Torres Strait Islander women (21% in a Darwin study) and those with a family history of PCOS or T2D.

PATHOLOGY

The pathological determinants of PCOS include hyperandrogenism and insulin resistance. Reproductive and metabolic features of PCOS are underpinned by insulin resistance (IR) which stimulates ovarian androgen production and decreases hepatic sex hormone-binding globulin (SHBG production) thus increasing total and free androgens.[10] Androgen abnormalities are present with 60-80% of women with PCOS showing higher concentrations of circulating free testosterone and other androgens.[6]

CLINICAL FEATURES

Women with PCOS present with diverse features including psychological (anxiety, depression, body image),[11-13] reproductive (irregular menstrual cycles, hirsutism, infertility and pregnancy complications)[14] and metabolic features (IR, metabolic syndrome, prediabetes, T2D and cardiovascular risk factors).[15,16] Presentation varies across the lifespan and between ethnicities.

PCOS AND T1D

While PCOS is traditionally associated with T2D and insulin resistance, there is evidence to suggest that PCOS prevalence is also increased in women with T1D. The pooled prevalence of PCOS was 24% in adolescent and adult women with T1D in a meta-analysis,[17] which is significantly higher than in women without diabetes (8–13%). In a recent large community-based study, the self-reported PCOS prevalence in Australian women of reproductive age with T1D was 14.2%, compared to 5.1% of non-diabetic controls[18] (unpublished data).

While the mechanisms for PCOS in T1D are still unclear, it has been hypothesised that supraphysiological doses of subcutaneous insulin are needed to reach the portal circulation in T1D, leading to exogenous hyperinsulinism. Exposure to excessive concentrations of insulin is thought to be the driver for ovarian-mediated androgen production, culminating in PCOS and hyperandrogenic traits.[17,19]

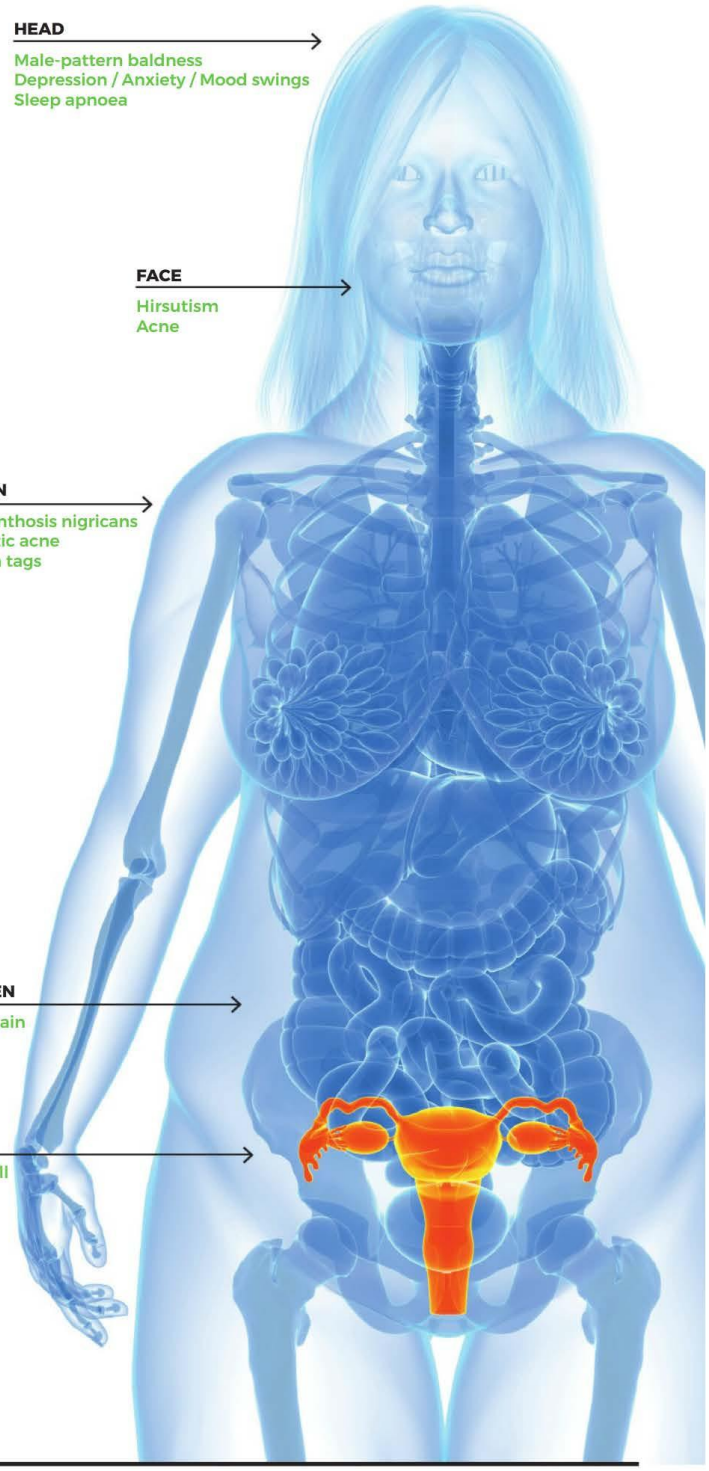
SCREENING FOR DYSGLYCAEMIA IN PCOS

The new PCOS guideline[20] recommends glycaemic assessment at baseline for all diagnosed with PCOS.

The assessment can be done using an oral glucose tolerance test (OGTT), fasting blood glucose or HbA1c. Of these, the guideline recommends an OGTT in presence of additional risk factors such as family history of diabetes, previous history of gestational diabetes, impaired fasting glucose or impaired glucose tolerance, high BMI (>25 kg/m^2, >23 kg/m^2 in Asians) and high risk

UNIQUE FEATURES OF
DIABETES MANAGEMENT IN PCOS COMPARED TO THE GENERAL POPULATION

- PCOS is underpinned by insulin resistance regardless of BMI. All women with PCOS, even if lean, are at increased risk of dysglycaemia.
- Women with PCOS have a 4 times higher risk of diabetes which manifests at a younger age compared with the general population.
- Lifestyle management is even more important, with higher rates of weight gain than in women without PCOS.
- Pre-conception counselling for weight and glycaemic management should be performed for all women with PCOS planning pregnancy.
- Women with PCOS should use appropriate contraception while taking a range of medications including GLP1 receptor agonists (for diabetes or weight loss) or spironolactone (for hirsutism).
- Use of metformin in pregnancy is still a question for further research. The authors do not recommend the use of metformin during pregnancy for PCOS management.



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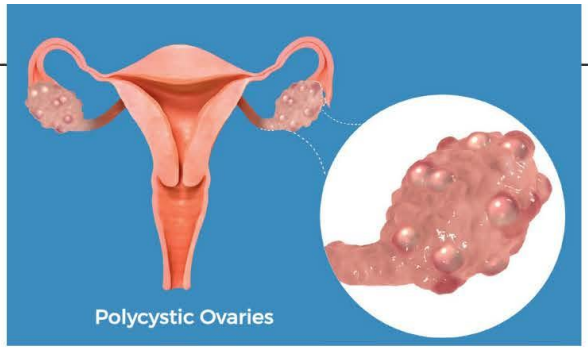

ethnicities. Follow up assessment can be done 1-3 yearly depending on risk factors. This risk of diabetes is independent of but exacerbated by obesity.[21]

An OGTT should also be recommended to all women with PCOS who are planning a pregnancy, or before 20 weeks' gestation if not performed pre-conception.[20] Pregnant women with normal glucose tolerance initially, should be offered a repeat OGTT at 24-28 weeks of gestation.

MANAGEMENT OF DYSGLYCAEMIA IN PCOS

Lifestyle modifications: The rate of obesity and weight gain is more in women with PCOS compared to those without PCOS.[22] Weight loss of just 5-10% is shown to significantly improve hormonal and metabolic features of PCOS including insulin resistance and glycaemic control.[21,23]

High BMI is often a major concern to women with PCOS,[24] and evidence suggests a lower quality of life (measured socially and psychologically) in those with obesity compared to those without.[24] Weight management, while important, is a sensitive subject that needs to be discussed, explaining the purpose and goals and asking permission beforehand.

The new guidelines recommend educating all women with PCOS regarding general principles of healthy eating and the importance of regular exercise. Monitoring weight is beneficial, aiming to prevent weight gain at first stage, particularly from adolescent age. A goal of 5-10% weight loss over 6 months is often achievable and leads to significant clinical improvement. No one diet has been shown to be advantageous over others in PCOS.

Regarding exercise, the guidelines recommend a minimum of 150 min/week of moderate intensity physical activity or 75 min/week of vigorous intensities or an equivalent combination of both in adults. There is a lack of evidence regarding the effect of exercise in lean women with PCOS.[25] However, some evidence in other populations suggest that insulin resistance would improve with regular exercise even without weight loss.[26,27]

Metformin: Metformin, an insulin sensitiser, is known to prevent or delay progression to diabetes in individuals with impaired glucose tolerance.[28] The new international guidelines recommend metformin for women with PCOS and impaired glucose tolerance or diabetes and those from high risk ethnicities. Although it is not a weight loss medication, metformin should also be offered to women with PCOS with BMI>25 kg/m^2, regardless of diabetes status to manage weight and metabolic outcomes,[29] where lifestyle modification does not address metabolic features.

Use of metformin in PCOS (without diabetes) is off-label on a private prescription, and the health professional should discuss the evidence, side effects (gastrointestinal and vitamin B12 deficiency) and elicit any concerns.[20] Gastrointestinal side effects are often dose dependent and can be self limiting.[30] We recommend a starting dose of 500mg daily with 500 mg increments every 1-2 weeks, to a tolerated dose of 1500mg daily. Extended release form of metformin may cause fewer side effects.

Bariatric surgery: Indications for bariatric surgery in the general population could be applied to women with PCOS who have a BMI ≥ 40 kg/m^2 or those with a BMI≥ 35 kg/m^2 and one or more obesity-related complication if non-surgical interventions fail and if pregnancy is not desired.

Bariatric surgery is shown to be beneficial for weight loss and diabetes management in the general population and in women with PCOS.[31,32] However, there is limited evidence regarding the effect on fertility and pregnancy outcomes and there are concerns regarding perinatal adverse effects including small for gestation babies and shorter gestation.[33] Pregnancy should be avoided for at least 12 months after bariatric surgery, using appropriate contraception.[20]

PCOS is a complex condition underpinned by insulin resistance. The PCOS guideline (2018) provides clear guidance on screening, prevention and management of diabetes risk in women with PCOS.

An extensive research translation program has been delieved to assist practitioners implement the PCOS guideline recommendations. To access the extensive range of practice tools and consumer resources, including the first evidence-based PCOS app, go to www.monash.edu/medicine/sphpm/mchri/pcos or search for "Monash PCOS".

The authors declares no conflict of interest. For references given in this article, please go to www.diabetesaustralia.com.au/diabetes-management-journal.


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